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HARVEY WASHINGTON WILEY, 1844–1930

HARVEY WASHINGTON WILEY

Born at Kent, Indiana, October 18, 1844.
Parents: Preston Pritchard and Lucinda Weir Maxwell Wiley.
Married Anna Campbell Kelton, February 27, 1911.
Children: Harvey Washington and John Preston.
Died June 30, 1930.
Buried with the veterans of the G. A. R., at Arlington Cemetery.

DEGREES

Hanover College: A.B., 1867; A.M., 1870; Ph.D., 1876; LL.D., 1898. Indiana Medical College: M.D., 1871; Harvard: B.S., 1873; Vermont: LL.D., 1911; Lafayette: Sc.D., 1912; Hahnemann Medical College: A.M., 1923.

POSITIONS

Corporal, Infantry Company I, 137th Regiment Indiana Volunteers, U. S. Army, 1864.
Professor of Latin and Greek, Butler University, 1868–70.
Teacher of Science, High School, Indianapolis, 1871.
Professor of Chemistry, Indiana Medical College, 1872–74.
Professor of Chemistry, Butler University, 1874.
Major, Military Staff of Governor A. G. Porter of Indiana, 1881.
Professor of Chemistry, Purdue University and State Chemist, Indiana, 1874–83.
Chief Chemist, U. S. Department of Agriculture, 1883–1912.
Professor of Agricultural Chemistry, George Washington University, 1899–1912.
Consulting Professor, Brooklyn Polytechnic Institute, 1905.
Redpath Chautauqua Lecturer, 1912–17.
Director, Bureau of Foods, Sanitation and Health, Good Housekeeping Magazine, 1912–1930.

MEMBER, JURY OF AWARDS

Columbian Exposition, 1893; Paris Exposition, 1900; Jamestown Exposition, 1907; Second International Congress of Refrigerating Industries, Vienna, 1910.

MEDALS AND DECORATIONS

Chevalier du Mérite Agricole, France, 1900; Physico-Chemical Academy, Italy, 1908; Chevalier Legion d'honneur, 1909; Elliott Cresson, Franklin Institute, 1910; Association of Official Agricultural Chemists, 1924.

HONORS

U. S. Representative: 3rd International Congress of Applied Chemistry, Vienna, 1898; 4th, Paris, 1900; 5th, Berlin, 1903; 6th, Rome, 1906; 7th, London, 1909; 8th, Washington, 1912; Société Universalle de la Croix blanche de Génève, 1912.

Honorary President: Association of Official Agricultural Chemists, 1912–30; also President, 1886 and Secretary, 1889–1912; First International Congress, Repression of Adulteration of Alimentary and Pharmaceutical Products, Geneva, 1908.

President: American Chemical Society, 1893–94; Indiana Academy of Science, 1902; U. S. Pharmacopeia Convention, 1910–20; American Therapeutic Society, 1911; Literary Society of Washington, 1910–1912; Industrial Academy.

Vice-President: American Association for the Advancement of Science, 1886; also Secretary of Council, 1890; General Secretary, 1891, and Emeritus Life Member, 1922; Washington Academy of Science, 1909; Society of Chemical Industry, 1910; Precieuse Ridicule.

Correspondent: Academician Section of Chemical Science, National University of La Plata, Argentine Republic, 1907.

Honorary Fellow: American Institute of Chemists, 1928.

Honorary Member: American Brewing Institute; British Federated Institute of Brewers; Franklin Institute; Philadelphia College of Pharmacy; Pennsylvania Pharmaceutical Association; Physico-Chemical Academy of Italy; Society of Public Analysts, London; Société science d'Hygiéne alimentaire.

Dr. Wiley was also a member of the American Medical Association, American Pharmaceutical Association, American Public Health Association, Indiana Academy of Science, Society of Biological Chemistry, the Philosophical Society of Washington, and Pi Gamma Mu (the National Society of Science).

BOOKS

Song Book, Association of Official Agricultural Chemists, 1891; Principles and Practice of Agricultural Analysis, 3 editions; 1001 Tests of Foods, Beverages and Toilet Accessories, 1914, 2 editions; Not by Bread Alone, the Principles of Human Nutrition, 1916; Health Reader—Physiology, Hygiene, 1916; Wiley Health Series, Books One, Two and Three, 1917; Foods and Their Adulteration, 3 editions, 1917; The Lure of the Land, 1919; Beverages and Their Adulterations, 1919; History of a Crime against the Food Law, 1929; Autobiography, 1930.

CLUBS

Cosmos (president, 1910–11), Chevy Chase, National Press, Harvard Union, Arts of Washington, and Chemists of New York.

WILEY—THE TEACHER

By W. W. Skinner

We are met with the sad realization that a silver thread binding the events of this Association is broken. It seems right and proper as our President has remarked, that we should at this accustomed hour pause in our deliberations and pay tribute to the memory of our former colleague and leader. Perhaps to most of us nothing that may be said about him will be new; certainly nothing that we may say will add one "jot or tittle" to the reputation or the fame of our departed leader. Rather is it an opportunity for us to formulate—feeble and inadequate though the attempt may be—a record of our esteem, our admiration and our love for Harvey W. Wiley. With this thought in mind the Committee has arranged this memorial, and it is proposed to gather in printed form the thoughts expressed here today, an appropriate photograph and a memorial tablet, and to bind them so that each of you may have a copy to place among the exhibits of your treasured memories.

To me has been assigned the honor of inaugurating these tributes. My tribute is to Wiley the teacher. This is indeed a peculiar privilege to one who received from the hands of Dr. Wiley a diploma conferring a graduate degree in agricultural chemistry earned by two years' study under his direction. Harvey W. Wiley was a great teacher of individuals, of classes, of masses. His greatest accomplishment perhaps was in teaching the general public, awakening in the public mind an appreciation of the application of science to the needs of everyday life. Some one has said that great teachers and great preachers are born, not made. This means that a certain few individuals are peculiarly and richly endowed by nature and are thus ordained to play a definite and important rôle in shaping the destiny of their fellow men by influencing and directing the thought of their own times as well as of that of generations yet unborn. A study of the pages of history will reveal that the great teachers of the world have had many similar mental characteristics, although each was influenced by the environment in which his lot was cast. Socrates, St. Paul, Luther, Wesley, Arnold, Gray, Agassiz, Johnson, Wiley,—all were marvelously equipped by nature with that something which modern psychologists term "the intelligence capacity," a necessary foundation upon which to place facts which, when properly integrated, produce a philosophy, the concrete products of which are genius and wisdom. Wiley had the opportunity and privilege of sitting at the feet of two of the great teachers I have named, Gray and Agassiz.

Concomitants of unusual intelligence capacity noted in all great teachers are an unquenchable craving for knowledge and a will to satisfy this capacity; an unbounded enthusiasm for the final accomplishment of anything undertaken; the ability and power to deduce from any given set of facts a conclusion which instantly becomes a conviction, with a characteristic flaming desire to bring all men to the same

degree of faith, a truly evangelical spirit which, if uncurbed by good judgment, may lead to bigotry; the gift of persuasion; the power to center and to hold the attention of men upon the specific thing to be desired; a love—an abiding love—for one's fellow man; truly the spirit of the Master, formulated in "thy neighbor as thyself."

All these attributes of a great teacher, and even others, were conferred by nature upon Harvey Washington Wiley. His marvelous capacity to absorb, to assimilate and to use information is illustrated by one small incident among many in my own personal experience. A hearing on an important controversial matter had been called for a certain Tuesday morning, and the opinion of the Chief of the Bureau of Chemistry was desired. Late Friday afternoon Dr. Haywood and I were called to the office, impressed with the importance of the matter, and told by Dr. Wiley what he wanted done. We worked feverishly all day Saturday and part of Sunday and Monday, combing the Department and Congressional Libraries for information and abstracting a large amount of literature, both foreign and domestic. About 2 P.M. Monday we went to the Doctor with our voluminous notes and a suitcase full of books of reference, but we were calmly advised that he was leaving for the Capitol and could not see us until the next day at 9 o'clock. The meeting was to be at 10:30. Promptly at 9 Haywood and I started to give the Doctor our data. He listened intently, interrupting with an occasional grunt or a pointed question, but he took no notes. At 10 o'clock we started for the meeting, not without considerable misgiving on the part of Haywood and me. When the time came for the Doctor to speak he presented the case in masterful fashion, citing foreign literature and other references to the discomfiture of the opponents. On the return to the Bureau, Haywood and I continued to marvel over his knowledge and ability.

A peculiar hand of destiny seems to have guided the fate of Harvey W. Wiley. Gathered around the table on numerous occasions at the annual conventions of this Association, some of us have had the privilege and pleasure of hearing from him the story of his early struggle for an education and the splendid instruction he received at Hanover College; the early determination to study and practice medicine; the acute disappointment at the failure of his first attempt to secure a teaching position; and how he later secured a position as teacher in a school in Lake County, Indiana, at the magnificent salary of $360 for the 6 months' term. Recounting these facts in his autobiography, he remarks about saving $100 of this first salary and sagely observes: "He thought then and still does that it is important to save a part of one's income no matter how small the income may be." The next year, 1868, we find the young Hoosier invited to become tutor of Latin and Greek at Northwestern Christian University, at Indianapolis (now Butler University), which position he held for three years. In the meantime he pursued his medical studies at the Indiana Medical College, receiving the M.D. degree in the spring of 1871, and soon after he was offered a position as teacher in the Central High School of Indianapolis. In the spring of 1872 he was elected to the Chair of Chemistry in the Indiana Medical School, later a part of the State University, but he accepted with the proviso that he should be granted a year's leave of absence to

study at Harvard to fit him for the job. This was the turning point in his career that was destined to divert his attention from medicine to chemistry. After his graduation from Harvard he returned to the Indiana Medical College, where he continued his profession of chemistry until 1875, when he was elected to the Chair of Chemistry at the young university about to be opened at Lafayette, Indiana, and now known as Purdue University. This position he held for nine years, until he was appointed Chief Chemist in the U. S. Department of Agriculture.

This teaching activity of fifteen years, through all the grades from the one-room rural school to the position of professor and head of the chemical department of a State University, was a gold mine of experience of just the kind to fit him for the rôle he was destined to play in arousing a national and an international public conscience regarding the importance of what to eat. During the greater part of his service in the Department of Agriculture he was teaching and preaching the gospel of Pure Food, and afterwards and until a few months of the end, on the public forum and in the public press, he continued to spread his gospel. Of the many monuments which he has left, perhaps that bestowed upon him by common consent, the "Father of Pure Food," will be most enduring.

The greatest accomplishment of Harvey W. Wiley, Teacher, however, in my opinion was not one of those heretofore noted—it was the benign influence which his kindly, humorous, infectious personality had upon the younger men with whom he came in contact. There are a number of men within the sound of my voice, and many, many more not present, whose lives and careers have been shaped and fashioned by contact with, and by the advice and influence of this great personality. These lives, these careers, are his greatest monuments.

It was a blessing that Dr. Wiley's great mental powers remained practically intact until the end. One of the most vivid memories I treasure of him was an experience one evening at the Cosmos Club shortly after his 82nd birthday, when, after a lecture in the Club Auditorium on the Einstein theory, a group was actively discussing the subject. Here, at the sunset of a life with fourscore years behind it, this brilliant, scintillating mind was participating in the discussion of this abstruse matter with an enthusiasm and acuteness quite equal to the best in the party, some with scarcely more than one and a half score years to their credit.

As his pupil, this feeble tribute—a mental wreath—I place upon a sarcophagus of my memory. To me Harvey Washington Wiley, the teacher, was the very embodiment of the thought expressed by his own beloved master, the great Agassiz, who exhorted his pupils:

Come wander with me
In regions yet untrod,
And read what is still unread
In the manuscript of God.

WILEY—THE CHEMIST

By C. A. Browne

The news of the passing of Dr. Wiley reached me in Leipzig on the fourth of last July while I was upon my way to visit the agricultural institute of that famous university city. Although not wholly unexpected, the tidings overwhelmed me with the sudden realization of a great personal loss for no one exercised so strong an influence as Dr. Wiley upon the shaping of my professional career. Many chemists in this audience can give similar testimony, for the part of Dr. Wiley in stimulating the activities of his fellow men was a most potent one. During my recent absence abroad I was impressed to note the extent of this influence even in foreign countries. Only the day previous to my learning of his death, one of the leading food chemists of Germany had spoken to me of the importance of his work. In Berlin I noticed his portrait upon the office wall of an internationally known sugar expert. In Paris I was told of the prominent part which he played in the reform of pure food legislation in France as a result of recommendations made at the request of the French government. Chemists in London spoke with pleasure of his visits to England and similar expressions of appreciation were made in Amsterdam, Vienna, Budapest and other cities which I visited. His influence then as a chemist was not simply local or national; it extended beyond the borders of his own country to other lands and peoples. He was known to them as to us not only for his work as a chemist but for his broad human interests and genial optimism—qualities which endeared him to all his fellow men irrespective of country or language or religion. Whole-souled, helpful, full of enthusiasm and a magnificent man among men—such was the characterization of him by a leading agricultural chemist of Holland.

The chemical activities of Dr. Wiley were of a varied character and pertained to agriculture, to the analysis of agricultural products, to agricultural technology and to problems of hygiene and the public welfare. His interests in agriculture and in agricultural technology were first awakened by his boyhood experiences upon an Indiana farm. Later his inclinations were given a special direction towards chemistry by his studies at Hanover College; his subsequent training in medicine at the Indiana Medical College marked the inception of his interest in the application of chemistry to questions of the public health. Post graduate studies were then undertaken at Harvard and also at Berlin, where he received his first instruction in advanced methods of food analysis more especially as a means of detecting adulteration. The results of these years of study were amplified by his work as Professor of Chemistry at Purdue University and as State Chemist of Indiana, when he first began to publish contributions upon new types of apparatus and upon the chemical analysis of foods.

These preliminary activities, however, served only as the preparation for the important work which Dr. Wiley was called upon to perform as chemist of the Department of Agriculture, an office which Commissioner Loring persuaded him to accept in 1883. His services in

this position were along three distinct lines of work, in each one of which he won the highest distinction.

The first of these services was his chemical study upon our sugar producing crops, the sorghum, the maple, the sugar cane and the sugar beet. His technological work upon the application of the diffusion process to the extraction of sugar from the cane will always rank as a classic for, although not introduced, his demonstration of its superiority over then existing methods was the effective stimulus which caused manufacturers to make a complete departure from the archaic type of cane mill that had remained unchanged for many generations. These experiments of Dr. Wiley, in the opinion of no less an authority than the late Dr. G. L. Spencer, marked the dawn of the modern cane sugar industry. Even more important was his work in determining the climatic boundaries within which the sugar beet can be grown successfully in the United States. It is a significant fact that nearly every successful beet-sugar factory in the United States is located within the belt which Dr. Wiley defined and that attempts to extend the cultivation of the beet to districts outside this zone have been attended with disaster. This work will stand as the best example of the climatic survey of a crop in the annals of American agricultural chemistry.

The second great contribution of Dr. Wiley to American chemistry was his work in standardizing and improving the methods of agricultural chemical analysis. He was a great student of this subject, devising many new pieces of apparatus and originating many new methods of procedure. His work in this field is best exemplified by his well-known *Principles and Practice of Agricultural Analysis* and by his work as a member of our Association. At a time when chemical analysis is so suffering from neglect in the modern curriculum that there is danger of its becoming a lost art it is well for us to bear in mind the determining part which this branch of our science played in Dr. Wiley's work of reform.

But chemical analysis with Dr. Wiley was only a means to the one important end which was the guiding star of his whole career, and this brings us to his third achievement as a chemist, namely, the contributions which he made to the public welfare. The analyses of American food products, which he instituted immediately after his appointment as chemist of the Department, and the results of which were finally published in that epoch-making series of publications known as Bulletin 13 of the Bureau of Chemistry, revealed an almost incredible state of adulteration and debasement. It was to the correction of these evils that he consecrated the remainder of his life. In the face of the most prolonged opposition by selfish commercial interests, he finally secured in 1906 the passage by Congress of the Food and Drugs Act, the crowning achievement of his career. Confronted with an even more determined resistance, he then began the administration of this act under difficulties which would have discouraged a less resolute reformer. The obstacles, the treachery, the abuse which he incurred in the discharge of his public duties at this time are so well-known that the story need not be told. The cause of pure food in America was fortunate in having as its first great protagonist a man of Dr. Wiley's courage and perseverance. His work in securing the passage of the Food and Drugs Act

will always rank as one of the outstanding accomplishments of American chemistry.

There are many other phases of Dr. Wiley's career as a chemist which might be enumerated. In his 29 years as chemist of the Department of Agriculture he built up an organization from six to more than six hundred employees. He originated many lines of chemical research in such fields as soils, milk products, road construction, and standardization of apparatus, that afterwards became the nuclei of separate bureaus which survive today as the offspring of his creative genius.

The career of Dr. Wiley is one of which we agricultural chemists may well be proud. His unwavering fortitude, his resistance to the selfish demands of commercialism, his unfailing optimism when confronted with almost insuperable difficulties and his sacrifice of private financial opportunities in order to serve the welfare of the people will always remain as shining examples for future generations of chemists.

WILEY—THE LEADER

By W. G. Campbell

Mass reform does not occur spontaneously. The ideas which have influenced civilization have been slow in their development. The civic, economic and moral betterment of the world has been effected only through prolonged and forceful advocacy.

The Federal Food and Drugs Act, an outstanding landmark in social advancement, is usually referred to as legislation enacted on a wave of popular approval. To conclude that this law was the outgrowth of a sudden revolt is erroneous. Recognition of the need for safeguarding the food supply is co-extensive with the progress of mankind. When our early progenitors found themselves in part able to apply their time to other vocations than the procurement of the day's food, occasion arose for the regulation of the operations of those to whom this important service was entrusted. Records of control by governmental function are as old as the Magna Charta. Throughout the ages attempts at adequate legislation occurred sporadically. The sum total of these efforts appraised so recently as at the beginning of the present century is represented by a number of provincial enactments, municipal ordinances and orders in council, woefully lacking in uniformity and thoroughly inadequate for the prevention of the most reprehensible forms of abuses.

When the Congress was asked first to consider the enactment of a Federal statute, food laws of a sort were being enforced by several of the States. This legislative proposal was not effectively advanced. Progress was slow. There was no organized support for the movement. Why? Surely the character of abuses which prevailed were not unknown. The work of public service and other laboratories had disclosed at least in part the sordidness of manufacturing practices. The debasement of elemental foods was not unusual. To a degree municipal control prevented the adulteration of milk by the addition of water, the removal of fat or the use of chemical preservatives. Knowledge of the

need for such control was general. By the same token the failure of the average food factory to maintain acceptable standards of cleanliness must have been known. Compulsory sanitary codes had public sanction. While accurate information was not available then, and in all instances may not be even now, of the refinement and control by which certain types of adulteration were developed, ample evidence had accumulated of long standing transgressions of the popular sense of honesty and decency. In a national way the public remained inactive. But local developments bore witness of a progressive widespread concern. Slowly, yet gradually, the idea of appropriate regulation of the production and distribution of foods and drugs was permeating the popular mind. With the inception of this view general reform was under way. To give to these growing convictions definite shape, to guide this ponderous movement, to translate sentiment into forceful action created alike the demand for, and the opportunity of, a leader.

The ability of a nation or an individual, or a cause, to survive is tested by the effectiveness with which emergencies are met. It is a gratifying historical fact that every stressful situation to which the American people has been subjected has produced a competent leader. The reform for purity in foods and drugs was no exception.

In Dr. Harvey W. Wiley there was presented one who led with the effectiveness of inspiration. His collegiate and vocational training testify to his interest in the public health. His public service career is characterized by the application of his own talent and that of the force which he directed to a disclosure of the truth about food and drug adulteration. Through experience he acquired superior knowledge. This fortified those inherent traits of leadership with which he was endowed—a wonderful intellect, a winning personality, dynamic forcefulness, and the eloquence to convert his listeners to the cause which he espoused. His leadership in the fight for legislation and subsequently in the early days of the enforcement of the measure which he advocated were featured by rugged honesty and a profound sincerity of interest in the public welfare.

Powerful opposition to the passage of the act existed. Eventually it was favorably considered by the more responsive branch of the Congress. It was repeatedly passed by one house, only to meet defeat in the other. Paternalism and progressivism were the textual qualities upon which political objection was based. In support, there was an overwhelming commercial protest against any form of control. In this compaign against determined and resourceful opposition there was begun a spectacular battle for public rights to which public support must be won. A program of education was begun. At no time in his distinguished career did Dr. Wiley demonstrate more convincingly his power of leadership than when engaged in molding public opinion favorable to this cause. Extensively and persuasively he voiced the message of his heart. He appealed for popular support on the text of consumer protection. He aroused the keenest interest throughout the country. He won first sympathy, then endorsement. Effectively marshalled, the public, ordinarily inarticulate, conveyed to Congress unmistakably its determination to have this bill passed. Reacting to this demand the Congressional vote in favor of the measure was so large that for practi-

cal purposes it may be said to have passed unanimously. But the quality of spontaneity was lacking. From the beginning of national agitation to the completion of legislative consideration, the ordeal was long-drawn and trying. That the movement culminated successfully when it did, and as it did, is due to the astute and determined leadership of Dr. Wiley, to the earnest and fervent quality of his advocacy. Among the leaders of all time for the cause of fair, upright, equitable commercial dealing he is illustrious.

Kind-hearted, patient and affable, he placed his trust in co-workers with that naïveté which won for him instantly the devotion and loyalty of supporters essential to successful leadership. The unequalled candor with which he dealt with all persons on all subjects acquired the respect alike of friends and foes. He was universally admired as an honest, public-spirited, fearless official. Having applied himself to the procurement of guarantees for the preservation of individual rights, he, as a friend to the consumer of food, a benefactor of mankind, will be remembered with increasing gratitude. As the leader, Martin Luther personified the movement of the Protestant Reformation in the sixteenth century. As the leader, Harvey W. Wiley personified the movement of food and drug reform in the twentieth century. He, through his sustained leadership, has won the undisputed right to the title "Father of our Food Law."

WILEY—THE PIONEER

By A. S. Mitchell

To appreciate the prominent part played by Dr. Wiley as a pioneer in the field of food standardization, it becomes necessary to consider the development of food legislation and standardization, both in this country and abroad.

Early food legislation was specific rather than general in character. It was designed to protect some particular class of product, or to prohibit some special form of adulteration. With the refinement of chemical, physical and microscopical methods for the examination of foods, legislation became broader in its scope. In this country the change began in "the 80's," and by 1895 many of the States had general food laws patterned somewhat upon the English law and containing many features that later were incorporated in the Federal Food and Drugs Act.

The early bulletins of the Division of Chemistry, particularly Bulletin 13, the first part of which was published in 1887, and the two special reports on "The Extent and Character of Food Adulteration," are evidences of Dr. Wiley's early interest in the subject of food adulteration and its control.

The reports of the proceedings of this association show that at the time of its inception, in 1884, fertilizers were its sole concern. In 1886 the scope of its activities was enlarged to include consideration of fertilizers, soils, cattle foods, dairy products, and other materials connected with agricultural industry. From then on we find mention of many food products, such as butter, cheese, grains, meals, fruit juices, wines, and jellies.

In 1896, for the first time, a special referee was appointed on food adulteration and in the report of that referee we find the first proposals for food standards. The report was approved, but hesitation was expressed with respect to the policy of endorsing standards. It was decided to submit the whole subject of food adulteration to a committee of five, such committee to have subcommittees and to refer methods and standards to the association at the following meeting. The incoming president, Dr. A. L. Winton, appointed a committee consisting of Dr. Wiley, Chairman; Prof. H. A. Weber, Ohio; Prof. M. A. Scovell, Kentucky; Dr. E. H. Jenkins, Connecticut; and Dr. William Frear, Pennsylvania, who was made Secretary.

The first meeting of the committee was held at the office of the Chief Chemist of the U. S. Department of Agriculture, Washington, D. C., January 28, 1898. There were present Messrs. Wiley, Scovell and Frear. Notwithstanding the scope of the committee, it was agreed to consider only the subject of food standards. (Methods for food analysis were later assigned to a special referee, with numerous subreferees, acting independently.)

Meanwhile Dr. Wiley had closely followed developments, both in this country and abroad, and was in touch with the work of leading analysts in Great Britain, Germany, Austro-Hungary and other European countries. He was familiar with the discussions appearing in the *Analyst*, the organ of the Society of Public Analysts of Great Britain, of which he was an honorary member. He knew of the studies being conducted by the Swiss analytical chemists not only on methods, but on standards for many of their products; of their conclusions, reached in 1890, concerning cacao products, and of the steps being taken toward the compilation of a Swiss food code. He knew of the proposals for an international agreement upon food adulteration, discussed at the International Congress of Food Chemists and Microscopists at Vienna in October, 1891, and of the action taken by the Austrian committee in 1892 for the preparation of the Codex Alimentarius Austriacus, a compilation which has become one of the most comprehensive of its kind.

No stenographic records of the meetings of Dr. Wiley's committee were kept, and it is from the carefully prepared notes of the secretary, Dr. Frear, that we are enabled to follow the details of its deliberations. Before attempting a decision upon policy, the whole subject of standardization was carefully canvassed, beginning with the history of the development of standards for weights and measures, monetary standards and coinage. No parallel was found to the formulation of standards for commodities until more recent times. In considering the necessity for standards it was reasoned on the one hand that variety in product is essential to meet the needs of life and varying purchasing powers of the consumers and that any system that would essentially narrow the variety of desirable merchandize would be contrary to public policy; that the immense numbers of commodities and the frequent changes in their nature as effected by supply and even by the dictates of fashion, would render the task of standardization difficult. On the other hand, what were tantamount to standards were being announced by State regulatory officials and fixed by State legislation; revenue laws, enacted without reference to the purity of the products involved, were acting to

fix the practices of the trades concerned; boards of trade, chambers of commerce, and produce exchanges, representing largely merchants as distinct from producers, were agreeing upon classifications for various products; and judicial decisions here and abroad, all were contributing toward more accurate classification of food products and a clearer distinction between related substances. Conflicting standards were being adopted, with embarrassing results, by States and communities having mutual relations. The need for authoritative standards was evident.

Standards must be considered in the light of analytical methods. In all countries the task of formulating food standards had devolved upon associations of chemists. Previous action on the part of this association had paved the way for a satisfactory review of the composition of many American food materials. Official methods for the determination of the normal constituents of the more important food stuffs, including dairy products, grains, sugar-house products, wines, etc., had been adopted. It was upon data obtained by these methods that American standards, for the most part, must be based. Dr. Wiley and his associates were convinced that the association should accept this responsibility.

A report was prepared setting forth at length the necessity for standards, and it was resolved that the adoption of standards by the association was desirable and that the committee, acting under the authority vested in it, proceed to secure the compilation of such standards. Special committees were appointed, and a plan of organization was drafted and approved by the entire committee. The more important food products were classified into related groups, and referees having special qualifications and experience in each group of products were selected among the members of the association. General instructions for the referees were prepared, and cooperation on the part of control officers and trade representatives was invited.

The referees began their work at once and in 1899 the association authorized the publication of the schedules of the committee, from time to time, as they might be perfected. In 1902 Congress inserted in the appropriations act for the Department of Agriculture a clause authorizing the Secretary of Agriculture to collaborate with this association in fixing standards for food products, and in the following year the Secretary was empowered to commission the members of the Standards Committee as special agents of the department.

From this brief recital it is apparent that Dr. Wiley endorsed the earliest requests for American food standards, kept abreast of food legislation and standardization here and abroad, took a leading part in perfecting the organization of the first committee on food adulteration, directed the activities of that committee toward standardization, served on each of the subcommittees, and, in the end, secured official recognition of the results of its labors. Truthfully may it be said that he was the father of American food standards and a pioneer in this branch of food chemistry.

WILEY—THE PUBLIC SERVANT

By W. D. Bigelow

For more than half a century Dr. Wiley was interested and active in public welfare, and for over a quarter of a century he served as an officer of that great army of men and women who spend their lives in Government employ.

As an agricultural chemist, his chief interest was in developing and promulgating information that would be advantageous to agriculture. His work on the study of food adulteration was begun before he went to the Department of Agriculture. While at Purdue University he secured a small appropriation from the State Legislature which made possible a systematic examination of the sugars and sirups then on sale in Indiana.

Early in his service of the Department of Agriculture this work was broadened and included a survey of the chief classes of foods on sale in the United States. His interest in this work lay in his belief that legislation was necessary in order to safeguard the health of consumers and to protect them from fraud. His publications of these investigations were for the purpose of making known to the public the extent to which adulteration and misbranding were prevalent.

Early in his official life he advocated legislation that would prevent as far as practicable the adulteration and misbranding of foods and drugs. He found that such legislation could only be enacted as a result of tremendous popular demand. Finding that that demand did not exist, he proceeded to create it; he was responsible, more than any other person, for the promulgation of information on which such sentiment must be based and for the development and organization of the insistent demand that finally led to the passage of the Food and Drugs Act. He diagnosed a public need and described that need in language so arresting that the public listened and believed and remembered. He was able to make his message first page news He was a publicist of supreme ability, but a publicist with the conscience and ethical standards of a scientist. He presented facts as he saw them without exaggeration or distortion.

Dr. Wiley's interest in public welfare and his activities in its behalf were not by any means limited to the sphere of his official life. He visited France at the request of the French government to advise that government with respect to controlling the purity of French wines. He was honorary president of the First International Congress for the Repression of Adulteration of Food and Drug Products which met in Geneva in 1908. He was the United States Representative of the Society of the White Cross of Geneva. He was President of the U. S. Pharmacopeial Convention from 1910 to 1920. Many other illustrations might be given of the scope of his interest and activities in matters relating to the public welfare.

He always maintained that the pure food law was enacted in the interest of the consumer and must be enforced in his interest. He also maintained that manufacturers of foods and drugs must adapt them-

selves to whatever measures were necessary for the protection of the interest of consumers.

As a Government official Dr. Wiley was interested in the broad field of agricultural chemistry, but his chief interest lay in the enactment and enforcement of the pure food law. He directed the activities of his Bureau primarily in the interest of the public welfare. He held that the law forbade the adulteration of foods and drugs and required that they be so labelled that consumers would not be deceived.

Having no court decisions as precedents to guide him, he considered it his duty to decide all doubtful questions in the interest of consumers in order that decisions might be secured regarding such of his actions as were not acceptable to the trade. The law in which he was so greatly interested is now administered by officials who began with Dr Wiley as young and inexperienced men. Their activities are guided in many respects by their twenty-four years' experience in the enforcement of this law and by numerous court decisions. The attitude they maintain in the enforcement of the law—the uniformity with which they consider all questions from the standpoint of the consumer—bears evidence that the principle which Dr. Wiley stressed most strongly has stood the test of time.

He insisted on strict observance of all laws and regulations insofar as they applied to the management of his Bureau. He would not tolerate the slightest variation from the requirements of the law with respect to appointments, the purchase of supplies or the disposal of property no longer needed. His policy in these matters built up an organization marked for efficiency and loyalty.

Dr. Wiley's character combined the essential qualities that make the good public servant. He had a fine respect for the law and also courage to apply it as rigorously to his own conduct and the conduct of his office as to outside interests. At the same time he was keen in his appreciation of the law's deficiencies and equally courageous in pointing out the need of their correction. He served the public not only by doing the thing that obviously had to be done, but also by helping the public to realize the further things that should be done. Perfunctory performance of prescribed duties was alien to his character.

WILEY—THE ORGANIZER (A.O.A.C.)

By H. A. Huston

This is an age of records. The front pages of the press are largely given over to reports of the record-breaking achievements of those who have gone the highest, or lowest, the fastest or slowest, and of those who have set new marks in all sorts of activities. Every one strives to be first in something, often without much consideration for its practical or cultural importance.

In connection with the friend whose distinguished career we are considering today, even I may lay claim to a modest record; for of all those present here today it was my good fortune to be the first to meet, know, live with, and work with Dr. Wiley.

At Purdue University, one month over fifty years ago, began a friendship, professional and social, that increased with the passing years, and which has been of very great value to me.

Forty-nine years ago there worked in Dr. Wiley's little private laboratory at Purdue three men who later on took an active part in the work of this Association: the late Dr. G. L. Spencer, the late Dr. C. A. Crampton, and myself. The equipment was limited, but our enthusiasm and appreciation of our generous and talented teacher were unbounded. Notwithstanding the fact that at that time Purdue was in dire financial distress, no fees or charges of any kind were required or accepted from those permitted to work in Dr. Wiley's private laboratory. He asked only character and the will to work.

But you may wonder what these reminiscences have to do with the work of Dr. Wiley as "The Organizer" in the A.O.A.C.

At that time he was already very keenly aware of the need for uniform and better methods of analysis for agricultural and food products. He had attended the meetings of those chemists who first came together to study and improve the methods of fertilizer analysis. A glance at the subjects of our theses may serve to illustrate how he began to organize us along this line. Spencer worked on the determination of phosphoric acid by the uranium method, Crampton on the separation and determination of the ingredients of commercial glucose, while I struggled with the influence of time and temperature on the estimation of reverted phosphoric acid—the beginning of a series of investigations, the reports of which must have sorely taxed the patience of those attending the meetings of the Association during the last decade of the past century.

Dr. Wiley's organization efforts in connection with the subjects that were to engross the attention of the A.O.A.C. began at Purdue and predated the organization of the Association itself.

When, in 1883, he became Chief Chemist of the U. S. Department of Agriculture, the factor of greatest weight in winning the appointment was his reputation as an authority on the production and analysis of sugars. From this time Dr. Wiley may fairly be said to have carried on almost single handed the essential organizing activities of the Association until he retired from the Department thirty years later.

Grant Allen, in one of his essays, asserts that the main qualification for a successful secretary of a scientific society is the ability to pick out the most satisfactory place for an outing. From this standpoint Dr. Wiley was a great organizing secretary, as will be heartily proclaimed by all those who were fortunate enough to take part in the delightful evenings at Marshall Hall during the earlier years of the Association's history.

As the Association year after year added subject to subject to its activities, Dr. Wiley could always be depended upon to give most valuable and cordial support to every new project. The growth in size of the printed *Methods of Analysis* of the Association from a little four-page folder to a closely printed volume of more than five hundred pages serves to indicate in one way the magnitude of the work of the central figure in the development of the Association. The great increase in membership and in attendance might serve as another measure, but any

standards of comparison of this kind, impressive though they be, must yield in importance to the earnestness and enthusiasm of Dr. Wiley in his efforts to benefit his fellow men. His long, arduous and fruitful career has already inspired many others to continue the work of this Association, whose members will always remember him as a most distinguished organizer and as a true friend.

WILEY—THE BOSS

By Mary Tidd Read

If I stuck to the subject assigned to me, my speech would be very short. Perhaps it wouldn't even be begun. There is little to be said of Dr. Wiley as a boss, because he never was a boss, in the sense in which that term is commonly used. He gave responsibilities, not orders, to his subordinates, and all worked together with one object in view, the boss working harder, longer hours and to better purpose than any of the others. Dr. Wiley dominated his people, not by rule and regulation —for he made none—but by the sheer force of a marvelous personality. Self-confident, believing thoroughly in the purposes he sought to achieve, he inspired his associates with his own zeal and made of the Bureau of Chemistry an organization to be reckoned with in the field of industry and in the scientific world.

Dr. Wiley's capacity for work was unlimited. He was also able to make other people work and like it. These traits, combined with his good nature and his infectious and constant flow of humor, made work days play days and service a pleasure.

When the food and drugs act finally passed both houses of Congress and was signed by President Roosevelt, there was great rejoicing in the Bureau of Chemistry, but I fear the great and glorious cause of humanity we had heard so much about was lost sight of, temporarily at least, in our enthusiasm over the fact that our boss had won a grand fight, and we had helped him do it.

To the public at large the "Father of the Pure Food Law" was a crusader, a champion, a man of parts; to us he was something greater than these. He was something so big and so fine, and so dear to us that it cannot be told from the platform or described by the written word. We recognized a leader. We loved the man.

No one who knew Dr. Wiley can think of him without recalling his fun. It was so spontaneous and such a vital part of him that to speak of him, even in the shadow of his death, just naturally brings to mind many instances of it. He was always in his merriest and most entertaining mood when gathered with friends around a banquet table. It has been said of him that to hear him talk of the necessity for a simple diet was an inspiration, but to see him eat a ten-course dinner was an exaltation. At a certain supper given in Washington, where Dr. Wiley was the principal speaker, the toastmaster facetiously introduced him as the star of the evening. The Doctor, just beginning his second helping, rose reluctantly and began—"The gentleman has made a slight mistake. I am not the star. I am the moon, and the fuller I am the brighter I shine."

Dr. Wiley walked to and from his office, a custom he continued after he left the Bureau of Chemistry and had offices in the Mills Building. Because of his failing eyesight and the ever increasing congestion of traffic, the habit was the source of much anxiety to his family and friends. Swinging down Connecticut Avenue of a morning, huge and hatless, he was a conspicuous and familiar figure in the crowd. There came a day when crossing an intersecting street he got all tangled up in the stream of passing motors, and saved himself from perhaps fatal injury only by his presence of mind and a quick leap to the curb. When he had landed and recovered his breath, he turned to an interested onlooker and blurted out, "There are only two kinds of people; the quick and the dead." I cannot vouch for the truth of this story, but it is a good one and bears all the earmarks of the Wiley wit. A prince of jokers himself, the Doctor was a conspicuous mark for other jokers. Many are the instances that might be cited did time permit.

The Doctor could even see the point of a joke when it was on him. At the recent meeting of the American Chemical Society, in Cincinnati, President McPherson made reference to the death of Dr. Wiley and the Council adopted appropriate resolutions. In one of these resolutions, it is stated: "During his presidency in 1893 and 1894 the American Chemical Society more than doubled its membership, greatly improved its journal, and received an impetus which has continued to the present time."

Dr. Wiley was keen to have the membership reach the thousand mark during his incumbency and launched a vigorous campaign to that end. That he succeeded is acknowledged in the resolution quoted, but there is one incident of that campaign that never went into the records of the Society and was known to very few outside the Bureau of Chemistry.

Prof. Hart, editor of the Journal at that time, thought it would be fitting to use the Doctor's picture for a frontispiece in the Journal for that year and wrote to the Bureau for a photograph that could be reproduced. We couldn't find one suitable for the purpose but volunteered to have a new one taken and forwarded in time for the December number of the Journal. To get a man's picture without his knowledge was a hard one, even for the Bureau of Chemistry. How it was accomplished with the help and connivance of Mr. Gilbert, the photographer, is quite another story. However, the picture was forwarded and appeared in due time. Of course, the Doctor was very much surprised and spent several very busy days protesting to skeptical friends that he used no pressure, financial or otherwise, to secure such an honor for himself. When the truth was finally told him, he owned that we had "put one over" on him and treated the entire Bureau to a royal repast.

The friends of the genial Chief of the Bureau of Chemistry were legion, and his loyalty to them withstood all the changes of the passing years. This loyalty is well illustrated in the following incident. One morning there appeared at the Bureau a small, white-haired, plainly dressed man who hesitatingly asked if he might see Dr. Wiley. He received scant attention from the snappy young messenger who showed him to a room where a United States Senator, an embassy attaché and several newspaper reporters were waiting their turn to speak to the

Chief. A few moments later, Dr. Wiley himself came into the room to look for a missing paper. The little man half rose from his chair but said nothing. The movement, however, attracted the Doctor's attention. He turned, took one look and with a broad grin of recognition gathered up the little man and swept him into the private office, while the prominent personages continued to wait with what patience they could command. The little man was W. W. Cheshire, county superintendent of schools, in Indiana, when young Harvey Wiley was trying to get a job to teach, in order to raise funds to permit him to pursue his medical studies. How Mr. Cheshire helped him to this accomplishment is feelingly told in the Doctor's autobiography.

Among the friends of Dr. Wiley in later years was a man whom perhaps some of you are old enough to remember: Dr. C. B. Dudley, of Altoona, chemist for the Pennsylvania Railroad Co. The two men were close friends for many years. Their careers ran in somewhat parallel lines. Both were soldiers in the Civil War, later on both became farmers and finally men of science. The death of Dr. Dudley, about 1908, brought a genuine sorrow to Dr. Wiley, and he very beautifully expressed his estimate of his friend in a sonnet he wrote at the time. The lines are so peculiarly applicable to Dr. Wiley, that with your permission I shall conclude with them.

Well have you done the labors of a life
Of service for your country and for God,
Whether the paths of flaming field you trod
In battle for the Nation rent with strife,
Where cannon's thunder smothered drum and fife,
Or following the plow across the sod,
Or tarrying where sons of science plod,
A fount of cheer for comrade, friend, and wife,
The ripple of your laugh, the clear sweet light
Of those dear eyes forever closed to earth,
Shall glad and guide me as I near the night
Now closing on my day of deeds and mirth,
Its glowing glory waxing ever bright
In th' unfathomed shadows of the second birth.

Dr. Wiley—A rare spirit, such as one meets but once in a lifetime. He has left a wealth of pleasant memories to a host of friends and admirers.

WILEY—THE MAN

By F. B. Linton

It was my very great privilege to work under Dr. Wiley in a close and confidential capacity from 1902, when he was 56, until 1912, when he was 66. At that time Dr. Wiley had reached the full height of his physical and mental powers. In those early days of food law enforcement, when precedent decisions were made and trails were blazed, a typical day with Dr. Wiley began at eight o'clock in the morning. For an hour he studied reports, correspondence and literature bearing upon any subjects under consideration. He dictated rapidly from nine until ten o'clock, clearing up a mass of correspondence.

There began at ten a series of conferences that would probably include a delegation of business men from New York City protesting

against some labeling requirement. He would certainly have a consultation with his chief inspector, Mr. W. G. Campbell, and with his first assistant, Dr. W. D. Bigelow. Representatives of the press were daily visitors, and Dr. Wiley always made it a point to see them, no matter how busy he might be. The French Ambassador, a personal friend, often called to enter an official protest against restrictions placed upon the importation of French peas, greened with copper sulfate, or upon other products from France, as did representatives of other foreign governments to protest against restrictions affecting importations from their respective countries. The Secretary of Agriculture sent for him frequently to discuss departmental matters. He might keep an appointment at the White House to demonstrate by actual experiment to President Roosevelt how imitation whisky could be manufactured on the spot by the mixture of certain well-known ingredients. At 3:00 o'clock in the afternoon he often took a train to New York, where he would attend a trade association banquet at 8:00 and deliver an hour's address which he had prepared going up on the train.

At midnight he took a train back to Washington, and the next day he went through somewhat the same routine, except that in the evening he might go out to Kensington to deliver an address before the Montgomery County Medical Association, return to the Cosmos Club at 11:00 o'clock, get into his evening clothes, go down to the New Willard to attend a charity ball, retire at 2:00 A.M., and call it a day's work. The next morning I would find him at his desk looking as pink and fresh as the rose he wore in the lapel of his coat.

Wit and humor with Dr. Wiley, as with Lincoln, were lubricants to reduce the friction that would otherwise have worn him out. Many an embarrassing situation was saved by his quick repartee. You perhaps have heard Dr. Wiley himself relate the incident of his visit to Girard College to make a formal address. He wore a high silk hat and a Prince Albert coat, and as he approached that institution he looked not unlike a Methodist bishop. At the gate a guard stopped him with, "I beg your pardon, sir, but Stephen Girard provided in his will donating the money for this institution that no minister of the gospel should ever be admitted to the building or grounds."

Dr. Wiley, drawing back with dignity exlaimed: "The hell you say!"

When the guard recovered sufficiently from the shock, he stepped aside and said, "Walk right in, sir, walk right in."

On one occasion when Dr. Wiley appeared before the House Committee on Agriculture to advocate an appropriation, Mr. Wadsworth, Chairman of the Committee, asked him to explain the meaning of the title "Agricultural Scientist," which had been frequently referred to in the hearings.

Dr. Wiley replied, "Mr. Chairman, an Agricultural Scientist is a scientist who can make two dollars grow on an appropriation bill where only one dollar grew before.

Mr. Wadsworth was apparently pleased with this reply for Dr. Wiley states in his autobiography that when the bill was reported to the House by the Committee, his salary was exactly doubled, which made it the maximum then paid to a bureau chief in the Department of Agriculture.

Dr. Wiley at one time presided at a debate held at the National Press Club for the entertainment and edification of the members of that club, their families and their guests. The debaters were two members of the House of Representatives and two United States Senators. A rigid time limit was placed upon each debater. Mr. Nicholas Longworth, now Speaker of the House, was one of the debaters. During the course of his remarks he was interrupted by members of the press club with interjections—sometimes pertinent, sometimes impertinent. Mr. Longworth finally turned to Dr. Wiley with the plea, "Mr. Chairman, I hope you will not charge these interruptions to my time."

Dr. Wiley replied, "Mr. Longworth, certainly I will charge the interruptions to your time. As a matter of fact the interruptions are much the best part of your speech."

Dr. Wiley was a big man—big in every sense of that word, big of hand, big of head, big of heart. He had vision to see, the courage to act. He feared no man, and he shrank from no difficulty. He has been pictured as the friend and confidant, and sometimes the opponent, of cabinet members, ambassadors and presidents of the United States. I would have you also see him as I saw him some ten years after he left the Department of Agriculture, at a little frame shack in Southwest Washington, attending the funeral services of an illiterate charwoman who years before had daily dusted his desk and scrubbed the floor of his office. I would have you see him as I saw him but a few months ago, at a negro church in Washington, attending the funeral services of a negro helper who, forty years before, had entered his laboratory to grind samples and do other manual work. When the negro minister in charge of the services unexpectedly called upon Dr. Wiley to say a few words, he rose to his feet and, addressing a congregation made up almost exclusively of negroes, paid a tribute in a few simple words to the faithful services of that negro helper that would rank as a classic in any forum of the world.

I first met Dr. Wiley on July 1, 1902, when he outlined my work. On July 2, 1930, just 28 years and one day later, it was my sad duty to stand for an hour by his casket as his body lay in state in the Vermont Avenue Christian Church, and a little later to follow it across Key Bridge to beautiful Arlington Cemetery where, in recognition of his services during the Civil War, he was buried with full military honors. As the squad fired the salute, as the bugle sounded taps, I could not quite visualize Dr. Wiley as a military man, though recognizing him as a good fighter. There came to my mind certain words of Robert Louis Stevenson's Morning Prayer, which seemed to me to express the spirit of Dr. Wiley, the man:

Give us to go blithely about our business. Help us to play the man; help us to perform the petty round of irritating concerns and duties with laughter and kind faces.

And his Evening Prayer:

Call us up with morning faces, and with morning hearts eager to be happy, if happiness shall be our portion; and if the day be marked for sorrow strong to endure it.

Before the meeting adjourned, the following letter was received from Mrs. Wiley and read by the president:

2345 Ashmead Place,
October 21, 1930.

Dr. E. M. Bailey and the members of the Association of Official Agricultural Chemists—

MY DEAR FRIENDS:—

I wish to take this earliest opportunity to tell you how touched Harvey Junior and I were by the noble tribute your Association paid to Dr. Wiley at this morning's session.

I need hardly tell you that in my belief, of the many organizations to which he belonged, it was the Association of Official Agricultural Chemists which was nearest the heart of my husband, and that it was your annual meeting each year in Washington toward which he looked with the keenest anticipations. Last year he grieved at his first enforced absence from your midst and only the visit of Dr. Brackett and Dr. Ross to our home reconciled him to his loss.

Of the many happy events in his long life none was more treasured than his eightieth birthday and the fortieth of the association, when you conferred upon him the beautiful medal epitomizing his life's work. I hope you will be glad to learn that the design upon your medal has been reproduced upon his monument at Arlington. It seemed fitting to me that this beautiful design conceived in the minds of his fellow chemists, his old friends and some of you, "his boys," should go above the old familiar title "Father of the Pure Food Law."

Dr. Wiley's body lies at Arlington, among that noble company of brave men who rest from their labors, but I feel sure that his militant spirit will go "marching on" in the hearts of the men who believed in him and who loved him.

Sincerely yours,

ANNA KELTON WILEY.

PROCEEDINGS OF THE FORTY-SIXTH ANNUAL CONVENTION OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1930

The forty-sixth annual convention of the Association of Official Agricultural Chemists was held at the Raleigh Hotel, Washington, D. C., October 20-22, 1930.

The meeting was called to order by the president, E. M. Bailey, Agricultural Experiment Station, New Haven Conn., on the morning of October 20, at 10:30 o'clock.

OFFICERS, COMMITTEES, REFEREES, AND ASSOCIATE REFEREES OF THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS FOR THE YEAR ENDING OCTOBER, 1931

President

H. D. HASKINS, Agricultural Experiment Station, Amherst, Mass.

Vice-President

A. E. PAUL, U. S. Food and Drug Adm., Chicago, Ill.

Secretary-Treasurer

W. W. SKINNER, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee

F. C. BLANCK, Washington, D. C.
J. W. KELLOGG, Harrisburg, Pa.
R. HARCOURT, Guelph, Can.
E. M. BAILEY, New Haven, Conn.

PERMANENT COMMITTEES

Recommendations of Referees

(Figures in parentheses refer to year in which appointment expires)

E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.), *Chairman*

SUBCOMMITTEE A: R. N. Brackett (1932), (Clemson College, S. C.), *Chairman;* H. H. Hanson (1934); H. R. Kraybill (1936). [Waters, brine, and salt; tanning materials and leathers; insecticides and fungicides (fluorine compounds); caustic poisons; soils and liming materials (reaction value of soils, liming materials, less common metals in soils); feeding stuffs (stock feed adulteration, mineral mixed feeds, moisture, biological methods for the determination of cod liver oil in feed mixtures); sugars and sugar products (honey, maple products, starch conversion products; drying, densimetric, and refractometric methods; polariscopic methods; chemical methods for reducing sugars); fertilizers (phosphoric acid, nitrogen, nitrogen activity methods, high analysis fertilizers, potash); plants (preparation of plant material for analysis, less common metals, total chlorine, carbohydrates); paints.]

SUBCOMMITTEE B: H. C. Lythgoe (1932), (Department of Public Health, Boston, Mass.), *Chairman;* A. G. Murray (1934); L. E. Warren (1936). [Specific gravity and alcohol, naval stores (rosin, turpentine); drugs (crude drugs, radioactivity in foods and drugs, emodin-bearing drugs, mercurials, microchemical methods for alkaloids, terpin hydrate, santonin, ether, thymol, small quantities of iodides in mixtures, bismuth compounds in tablets, phenolsulfonates, sulfonal and trional, guaiacol, iodoform, belladonna ointment and stramonium ointment, bromide-bromate methods; ipomea, jalap and podophyllum, solution of dextrose in ampoules, rhubarb and rhaphonticum, calcium gluconate, tetrachlorethylene); beers, wines, and distilled liquors.]

SUBCOMMITTEE C: J. O. Clarke (1932), U. S. Food and Drug Administration, Chicago, Ill.), *Chairman;* G. G. Frary (1934); H. A. Lepper (1936). [Dairy products (milk, butter, cheese, malted milk, dried milk, ice cream, milk proteins, qualitative tests); fats and oils; baking powder and baking chemicals; eggs and egg products (reducing sugars, sucrose, added salt and glycerol; fat lipoids, lipoid P_2O_5 and total P_2O_5, detection of decomposition, water-soluble protein, unsaponifiable matter and ash); food preservatives, (formic acid in sugars and sugar products), coloring matters in foods, metals in foods (arsenic, boron, tin, copper and zinc, and lead), fruits and fruit products (soluble solids, ash, fruit acids, effect of H-ion concentration on extraction of fruits), canned foods, vinegars, flavors and non-alcoholic beverages, meats and meat products (separation of meat proteins), gelatin, cacao products (crude fiber, cacao butter, sucrose and lactose in milk chocolate), coffee, spices and other condiments, cereal foods (ash in flour and in alimentary paste and ash and chlorine in baked products, H-ion concentration of flour, diastatic value of flour, starch in flour, flour-bleaching chemicals, foreign methods for testing flour, methods for alimentary paste, bread and baked products—(a) sampling and determination of moisture, (b) lipoids, lipoid P_2O_5, total P_2O_5 and fat, (c) crude fiber, milk solids in milk bread, rye flour in rye bread, experimental baking tests, unsaponifiable matter in flour and in alimentary pastes and water-soluble protein in alimentary pastes).]

Committee to Cooperate with Other Committees on Food Definitions

C. D. HOWARD (Board of Health, Concord, N. H.), *Chairman*
E. M. BAILEY G. G. FRARY

EDITORIAL COMMITTEE

W. W. SKINNER, *General Chairman*

Journal

R. B. DEEMER (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman* (1931)

H. R. KRAYBILL (1930) F. C. BLANCK (1933)
W. F. HAND (1932) W. S. FRISBIE (1934)
MARIAN E. LAPP, *Associate Editor*

Methods of Analysis

W. W. SKINNER, *Chairman*

J. A. LECLERC L. E. WARREN
J. W. SALE MARIAN E. LAPP
G. G. FRARY

Principles and Practice of Agricultural Analysis

C. A. BROWNE (Bureau of Chemistry and Soils, Washington, D. C.), and W. W. SKINNER

SPECIAL COMMITTEES

Committee on Definition of Terms and Interpretation of Results on Fertilizers and Liming Materials

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FREDERICK BATES (Bureau of Standards, Washington, D. C.), *Chairman*

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H. J. PATTERSON, College Park, Md.
W. H. MACINTIRE, Knoxville, Tenn.

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F. C. BLANCK: *Foods*
J. W. KELLOGG: *Feeding Stuffs*
A. E. PAUL: *Drugs*
A. G. MCCALL: *Soils and Liming Materials*
C. C. MCDONNELL: *Insecticides and Fungicides*
R. N. BRACKETT: *Fertilizers*
F. W. ZERBAN: *Saccharine Products*
R. W. FREY: *Tanning Materials and Leathers*
J. W. SALE: *Water*
A. J. PATTEN: *Plants*

Committee on Bibliography

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F. P. VEITCH

Committee on Necrology

C. A. BROWNE (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman*
W. F. HAND H. C. LYTHGOE

REFEREES AND ASSOCIATE REFEREES

WATERS, BRINE, AND SALT:
General referee: C. H. Badger, Food and Drug Adm., Washington, D. C.

TANNING MATERIALS AND LEATHERS:
General referee: I. D. Clarke, Bureau of Chemistry and Soils, Washington, D. C.

INSECTICIDES AND FUNGICIDES:
General referee: J. J. T. Graham, Food and Drug Adm., Washington, D. C.

FLUORINE COMPOUNDS:
Associate referee: G. A. Shuey, Agricultural Experiment Station, Knoxville, Tenn.

CAUSTIC POISONS:
General referee: J. J. T. Graham

SOILS AND LIMING MATERIALS:
General referee: W. H. MacIntire, Agricultural Experiment Station, Knoxville, Tenn.

REACTION VALUE OF SOILS:

a. ALKALINE SOILS:
Associate referee:

b. ACID SOILS:
Associate referee: E. T. WHERRY, University of Pennsylvania, Philadelphia, Pa.

LIMING MATERIALS:
Associate referee: W. M. Shaw, Agricultural Experiment Station, Knoxville, Tenn.

LESS COMMON METALS IN SOILS:
Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

FEEDING STUFFS:
General referee: V. E. Munsey, Food and Drug Adm., Washington, D. C.

STOCK FEED ADULTERATION:
Associate referee: H. E. Gensler, Department of Agriculture, Harrisburg, Pa.

MINERAL MIXED FEEDS:
Associate referee: H. A. Halvorson, Old Capitol Building, St. Paul, Minn.

MOISTURE:
Associate referee: G. E. Grattan, Department of Agriculture, Ottawa, Canada

BIOLOGICAL METHODS FOR THE DETERMINATION OF COD LIVER OIL IN FEED MIXTURES:
Associate referee: W. B. Griem, Department of Agriculture and Markets, Madison, Wis.

SUGARS AND SUGAR PRODUCTS:
General referee: R. T. Balch, Bureau of Chemistry and Soils, Washington, D. C.

HONEY:
Associate referee: H. A. Schuette, University of Wisconsin, Madison, Wis.

MAPLE PRODUCTS:
Associate referee: J. F. Snell, Macdonald College, Quebec, Canada

STARCH CONVERSION PRODUCTS:
Associate referee:

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS:
Associate referee: C. F. Snyder, Bureau of Standards, Washington, D. C.

POLARISCOPIC METHODS:
Associate referee: S. Byall, Bureau of Chemistry and Soils, Washington, D. C.

CHEMICAL METHODS FOR REDUCING SUGARS:
Associate referee: R. F. Jackson, Bureau of Standards, Washington, D. C.

FERTILIZERS:
General referee: G. S. Fraps, Agricultural Experiment Station, College Station, Tex.

PHOSPHORIC ACID:
Associate referee: W. H. Ross, Bureau of Chemistry and Soils, Washington, D. C.

NITROGEN:
Associate referee: A. L. Prince, Agricultural Experiment Station, New Brunswick, N. J.

HIGH ANALYSIS FERTILIZERS:
Associate referee: J. B. Smith, Agricultural Experiment Station, Kingston, R. I.

POTASH:
Associate referee: L. D. Haigh, Agricultural Experiment Station, Columbia, Mo.

PLANTS:
General referee: O. B. Winter, Agricultural Experiment Station, E. Lansing, Mich.

PREPARATION OF PLANT MATERIAL FOR ANALYSIS:
Associate referee: H. R. Kraybill, Agricultural Experiment Station, Purdue, Ind.

LESS COMMON METALS:
Associate referee: J. S. McHargue, Agricultural Experiment Station, Lexington, Ky.

TOTAL CHLORINE:
Associate referee: M. F. Mason, Agricultural Experiment Station, E. Lansing, Mich.

CARBOHYDRATES:
Associate referee: J. T. Sullivan, Agricultural Experiment Station, Purdue, Ind.

FORMS OF NITROGEN:
Associate referee: H. B. Vickery, Agricultural Experiment Station, New Haven, Conn.

PAINTS:
General referee: C. S. Ladd, Office of Food Commissioner and Chemist, Bismarck, N. D.

SPECIFIC GRAVITY AND ALCOHOL:
General referee: A. W. Hanson, Food and Drug Adm., Minneapolis, Minn.

NAVAL STORES:
General referee and associate referee on rosin: F. P. Veitch, Bureau of Chemistry and Soils, Washington, D. C.

TURPENTINE:
Associate referee: V. E. Grotlisch, Food and Drug Adm.. Washington, D. C.

DRUGS:
General referee: A. E. Paul, 1625 Transportation Bldg., Chicago, Ill.

CRUDE DRUGS:
Associate referee: H. W. Youngken, Massachusetts College of Pharmacy, Boston, Mass.

RADIOACTIVITY IN DRUGS AND WATER:
Associate referee: J. W. Sale, Food and Drug Adm., Washington, D. C.

EMODIN-BEARING DRUGS:
Associate referee: E. O. Eaton, Food and Drug Adm., San Francisco, Calif.

MERCURIALS:
Associate referee: T. F. Pappe, Food and Drug Adm., Chicago, Ill.

MICROCHEMICAL METHODS FOR ALKALOIDS:
Associate referee: C. K. Glycart, Food and Drug Adm., Chicago, Ill.

TERPIN HYDRATE:
Associate referee: C. B. Stone, Food and Drug Adm., Minneapolis, Minn.

SANTONIN:
Associate referee: H. M. Burlage, School of Pharmacy, Purdue University, West Fayette, Ind.

ETHER:
Associate referee: Leslie Hart, Food and Drug Adm., St. Louis, Mo.

THYMOL:
Associate referee: F. L. Hart, Food and Drug Adm., St. Louis, Mo.

SMALL QUANTITIES OF IODIDES IN MIXTURES:
Associate referee: H. B. Mead, Food and Drug Adm., New York City.

BISMUTH COMPOUNDS IN TABLETS:
Associate referee: J. Calloway, Jr., Food and Drug Adm., New York, City.

PHENOLSULFONATES:
Associate referee: E. H. Grant, Food and Drug Adm., Baltimore, Md.

SULFONAL AND TRIONAL:
Associate referee: W. S. Hubbard, Schwarz Labs., New York City.

GUAIACOL:
Associate referee: N. T. Knight, Room 204, Old Custom House, St. Louis, Mo.

IODOFORM:
Associate referee: W. F. Kunke, Food and Drug Adm., Chicago, Ill.

BELLADONNA OINTMENT AND STRAMONIUM OINTMENT:
Associate referee: E. C. Deal, Food and Drug Adm., New Orleans, La.

BROMIDE-BROMATE METHODS:
Associate referee: L. E. Warren, Food and Drug Adm., Washington, D. C.

IPOMEA, JALAP AND PODOPHYLLUM:
Associate referee: L. E. Warren

SOLUTION OF DEXTROSE IN AMPOULES:
Associate referee: F. C. Sinton, Food and Drug Adm., Chicago, Ill.

RHUBARB AND RHAPHONTICUM:
Associate referee: Arno Viehoever, College of Pharmacy, Philadelphia, Pa.

CALCIUM GLUCONATE:
Associate referee: H. J. Fisher, Agricultural Experiment Station, New Haven, Conn.

TETRACHLORETHYLENE:
Associate referee: F. L. Elliott, Food and Drug Adm., Baltimore, Md.

DAIRY PRODUCTS:
General referee: H. C. Lythgoe, Department of Public Health, Boston, Mass.

MILK:
Associate referee: H. Hoffmann, Jr., Dairy and Food Department, St. Paul, Minn.

BUTTER:
Associate referee: C. W. Harrison, Food and Drug Adm., Minneapolis, Minn.

CHEESE:
Associate referee: C. B. Stone, Food and Drug Administration, Minneapolis, Minn.

MALTED MILK:
Associate referee: F. Hillig, Food and Drug Adm., Washington, D. C.

DRIED MILK
Associate referee: E. L. P. Treuthardt, Food and Drug Adm., Boston, Mass.

ICE CREAM:
Associate referee: G. G. Frary, Department of Agriculture, Vermilion, S. D.

MILK PROTEINS:
Associate referee: M. L. Offutt, Food and Drug Adm., New York City.

QUALITATIVE TESTS:
Associate referee: K. E. Wright, Dept. Animal and Dairy Husbandry, Amherst, Mass.

FATS AND OILS:
General referee: G. S. Jamieson, Bureau of Chemistry and Soils, Washington, D. C.

BAKING POWDERS AND BAKING CHEMICALS:
General referee: W. C. Geagley, Department of Agriculture, Lansing, Mich.

EGGS AND EGG PRODUCTS:
General referee: S. Alfend, Food and Drug Adm., St. Louis, Mo.

REDUCING SUGARS, SUCROSE, ADDED SALT AND GLYCEROL:
Associate referee: S. Alfend.

FAT, LIPOIDS, LIPOID P_2O_5 AND TOTAL P_2O_5:
Associate referee: J. H. Bornmann, Food and Drug Adm., Chicago, Ill.

DETECTION OF DECOMPOSITION:
Associate referee: H. D. Grigsby, Food and Drug Adm., New York City.

WATER-SOLUBLE PROTEIN, UNSAPONIFIABLE MATTER, AND ASH:
Associate referee: L. C. Mitchell, Food and Drug Adm., Chicago, Ill.

FOOD PRESERVATIVES:
General referee: J. C. Krantz, Jr., State Department of Health, Baltimore, Md.

FORMIC ACID IN SUGARS AND SUGAR PRODUCTS:
Associate referee: F. W. Zerban, Sugar Trade Lab., New York City.

SULFUROUS ACID IN DRIED FRUITS:
Associate referee: P. F. Nichols, Agricultural Experiment Station, Berkeley, Calif.

COLORING MATTERS IN FOODS:
General referee: C. F. Jablonski, Food and Drug Adm., New York City.

METALS IN FOODS:
General referee: G. C. Spencer, Bureau of Chemistry and Soils, Washington, D. C.

ARSENIC:
Associate referee: W. C. Taber, Food and Drug Adm., San Francisco, Calif.

BORON:
Associate referee: G. C. Spencer.

TIN:
Associate referee: A. E. Mix, Food and Drug Adm., Washington, D. C.

COPPER AND ZINC
Associate referee: Reed Walker, Bureau of Chemistry and Soils, Washington, D. C.

LEAD:
Associate referee: W. J. McCarthy. Food and Drug Adm., Cincinnati, Ohio.

FRUITS AND FRUIT PRODUCTS:
General referee: H. J. Wichmann, Food and Drug Adm., San Francisco, Calif.

SOLUBLE SOLIDS:
Associate referee: L. H. McRoberts, Food and Drug Adm., San Francisco, Calif.

ASH:
Associate referee: H. J. Wichmann.

FRUIT ACIDS:
Associate referee: B. G. Hartmann, Food and Drug Adm., Washington, D. C.

EFFECT OF H-ION CONCENTRATION ON EXTRACTION OF FRUITS:
Associate referee: L. A. Salinger, Food and Drug Adm., San Francisco, Calif.

CANNED FOODS:
General referee: V. B. Bonney, Food and Drug Adm., Washington, D. C.

VINEGARS:
General referee: A. M. Henry, Food and Drug Adm., Philadelphia, Pa.

FLAVORS AND NON-ALCOHOLIC BEVERAGES:
General referee: J. B. Wilson, Food and Drug Adm., Washington, D. C.

MEATS AND MEAT PRODUCTS:
General referee: R. H. Kerr, Bureau of Animal Industry, Washington, D. C.

SEPARATION OF MEAT PROTEINS:
Associate referee: W. S. Ritchie, University of Missouri, Columbia, Mo.

GELATIN:
General referee: R. M. Mehurin, Bureau of Animal Industry, Washington, D. C.

CACAO PRODUCTS:
General referee: J. W. Sale, Food and Drug Adm., Washington, D. C.

CRUDE FIBER:
Associate referee: Marie L. Offutt, Food and Drug Adm., New York City.

CACAO BUTTER:
Associate referee: W. O. Winckler, Food and Drug Adm., Washington, D. C.

SUCROSE AND LACTOSE IN MILK CHOCOLATE:
Associate referee: Jacob Fitelson, Food and Drug Adm., New York City.

COFFEE:
General referee: P. A. Clifford, Food and Drug Adm., Washington, D. C.

GUMS IN FOODS:
General referee: L. J. Cross, Dept. of Dairy Ind. Agr. College, Ithaca, N. Y.

SPICES AND OTHER CONDIMENTS:
General referee: H. A. Lepper, Food and Drug Adm., Washington, D. C.

CEREAL FOODS:
General referee: J. A. LeClerc, Bureau of Chemistry and Soils, Washington, D. C.

ASH IN FLOUR AND IN ALIMENTARY PASTE AND ASH AND CHLORINE IN BAKED PRODUCTS:
Associate referee: D. A. Coleman, Bureau of Agricultural Economics, Washington, D. C.

H-ION CONCENTRATION OF FLOUR:
Associate referee: Emily Grewe, Bureau of Dairy Industry, Washington, D. C.

DIASTATIC VALUE OF FLOUR:
Associate referee: M. J. Blish, Agricultural Experiment Station, Lincoln, Nebr.

STARCH IN FLOUR:
Associate referee: Lewellyn Jones, Food and Drug Adm., Kansas City, Mo.

FLOUR-BLEACHING CHEMICALS:
Associate referee: Dorothy Scott, Food and Drug Adm., New York City.

FOREIGN METHODS FOR TESTING FLOUR:
Associate referee: C. H. Bailey, University of Minnesota, Minneapolis, Minn.

CO_2 IN SALT-RISING FLOUR:
Associate referee: L. D. Whiting, Ballard and Ballard, Louisville, Ky.

METHODS FOR ALIMENTARY PASTE, BREAD AND BAKED PRODUCTS:

a. SAMPLING AND DETERMINATION OF MOISTURE:
Associate referee: L. H. Bailey, Bureau of Chemistry and Soils, Washington, D.C.

b. LIPOIDS, LIPOID P_2O_5, TOTAL P_2O_5 and FAT:
Associate referee: J. H. Bornmann, Food and Drug Adm., Chicago, Ill.

c. CRUDE FIBER:
Associate referee: R. L. Horst, Food and Drug Adm., New Orleans, La.

MILK SOLIDS IN MILK BREAD:
Associate referee: L. H. Bailey

RYE FLOUR IN RYE BREAD:
Associate referee: M. R. Coe, Food and Drug Adm., Washington, D C.

EXPERIMENTAL BAKING TESTS:
Associate referee: C. C. Fifield, Bureau of Agricultural Economics, Washington, D. C.

UNSAPONIFIABLE MATTER IN FLOUR AND IN ALIMENTARY PASTES AND WATER-SOLUBLE PROTEIN IN ALIMENTARY PASTES:
Associate referee: L. C. Mitchell, Food and Drug Adm., Chicago, Ill.

BEERS, WINES, AND DISTILLED LIQUORS:
General referee: W. V. Linder, Bureau of Internal Revenue, Washington, D. C.

MEMBERS AND VISITORS PRESENT, 1930 MEETING

Alexander, L. T., Bureau of Chemistry and Soils, Washington, D. C.
Alfend, S., Food and Drug Administration, St. Louis, Mo.
Allen, C. D., H. Kohnstamm & Co., New York City
Allison, F. E., Bureau of Chemistry and Soils, Washington, D. C.
Almy, L. H., H. J. Heinz Co., Pittsburgh, Pa.
Anderson, E. L., Food and Drug Administration, Baltimore, Md.
Anderson, M. S., Bureau of Chemistry and Soils, Washington, D. C.
Atwater, C. G., The Barrett Co., Raleigh, N. C.

Badger, C. H., Food and Drug Administration, Washington, D. C.
Bailey, E. M., Agricultural Experiment Station, New Haven, Conn.
Bailey, L. H., Bureau of Chemistry and Soils, Washington, D. C.
Bainbridge, W. C., H. Kohnstamm & Co., New York City
Balch, R. T., Bureau of Chemistry and Soils, Washington, D. C.
Barbella, N. G., Food and Drug Administration, Washington, D. C.
Barnard, H. E., Indianapolis, Ind.
Bates, M. A., Bureau of Chemistry and Soils, Washington, D. C.
Batton, H. C., Swift & Co., Baltimore, Md.
Baumgardner, R. E., Frederick, Md.
Beal, W. H., Office of Agricultural Experiment Stations, Washington, D. C.
Beeson, K. C., Bureau of Chemistry and Soils, Washington, D. C.
Berry, C. R., State Department of Agriculture, Richmond, Va.
Beyer, G. F., Prohibition Bureau, Washington, D. C.
Bidwell, G. L., Food and Drug Administration, Washington, D. C.
Bigelow, W. D., Research Laboratories, Washington, D. C.
Blackwell, A. T., Central Chemical Co., Baltimore, Md.
Bonney, V. B., Food and Drug Administration, Washington, D. C.
Bopst, L. E., College Park, Md.
Bornmann, J. H., Food and Drug Administration, Chicago, Ill.
Boyle, M., Food and Drug Administration, Washington, D. C.
Brackett, R. N., Clemson College, S. C.
Bradford, Z. B., Department of Agriculture, Raleigh, N. C.
Broughton, L. B., University of Maryland, College Park, Md.
Breckenridge, J. E., American Agricultural Chemical Co., Woodbridge, N. J.

Britton, L. E., 40 N. Market St., Boston, Mass.
Broll, H. R., City Health Department, Baltimore, Md.
Brown, B. E., Bureau of Chemistry and Soils, Washington, D. C.
Browne, C. A., Bureau of Chemistry and Soils, Washington, D. C.
Buic, T. S., Tower Bldg., Washington, D. C.
Burdick, C. L., Du Pont Ammonia Corp., Wilmington, Del.
Burritt, Loren, Prohibition Bureau, Washington, D. C.
Butt, C. A., International Agricultural Corporation, Atlanta, Ga.

Callaway, J., Food and Drug Administration, New York City
Callister, G. F., N. V. Potash Export My., New York City
Campbell, W. G., Food and Drug Administration, Washington, D. C.
Capen, R. G., Bureau of Chemistry and Soils, Washington, D. C.
Carpenter, F. B., Virginia-Carolina Chemical Corporation, Richmond, Va.
Carroll, J. S., N. V. Potash Export My., Jackson, Miss.
Carter, R. H., Bureau of Chemistry and Soils, Washington, D. C.
Casey, F. W., Department of Agriculture, Washington, D. C.
Cathcart, C. S., Agricultural Experiment Station, New Brunswick, N. J.
Charlton, R. C., American Agricultural Chemical Co., Baltimore, Md.
Chesnut, V. K., Food and Drug Administration, Washington, D. C.
Claman, S., State Department of Health, Baltimore, Md.
Clark, A. W., Agricultural Experiment Station, Geneva, N. Y.
Clarke, I. D., Bureau of Chemistry and Soils, Washington, D. C.
Clarke, J. O., Food and Drug Administration, Chicago, Ill.
Clevenger, J. F., Food and Drug Administration, New York City
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PRESIDENT'S ADDRESS

TWO PIONEERS IN AGRICULTURAL CHEMISTRY OF PARTICULAR INTEREST TO THE ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS

By E. M. BAILEY (Agricultural Experiment Station, New Haven, Conn.)

Although the duties, rights and privileges of the president of this association as set forth in the constitution do not include the making of a presidential address, the custom has continued so long that it appears to have acquired the status of an unwritten by-law. But whatever the criticisms that may attach to you for thus indulging your presiding officers in the past, it cannot be denied, when we recall even those addresses which fall within the scope of our own recollections, that they have been at once enjoyable, helpful and stimulating. And so in the light of the results which have followed your leniency, your offense, if there be any, is largely mitigated, if not entirely pardoned.

In a few years this association will have completed a half century of useful and highly important public service. In that period its activities have grown as the everincreasing applications of chemistry to agriculture have been recognized and put into operation. The scope of our work is now enormously expanded as compared with that which absorbed the attention of the founders of this body, but their ideals have ever characterized the association's endeavors. We glory in our long record of successful accomplishment and in the lives of the many distinguished members who have contributed so conspicuously to that success.

Reflecting upon the honor which you have graciously bestowed upon me, I am reminded that the experiment station which I represent has now for the fifth time been honored in having a member of its staff serve in the capacity of president of this association. Whether or not this record is unique, it nevertheless brings to us at the Station a feeling of deep appreciation and that satisfaction which comes from the consciousness of sustained effort in furthering the aims and purposes to which our association is dedicated.

In choosing a theme that would be worthy of your attention for a brief while today, I have been guided by several compelling circumstances. The first of these is that Professor Samuel W. Johnson, who perhaps more than any other man was responsible for the introduction of the agricultural experiment station idea in this country, was the first president of this association. The second circumstance is that during the past year I have had occasion to review the career of John Pitkin Norton, pioneer in chemical education in the United States, a man particularly devoted to the develop-

ment of agricultural chemistry, and the teacher, counselor and friend of Professor Johnson. The development of chemistry as an aid to the advancement of agriculture is closely identified with the careers of these two men; moreover, their ideals are reflected in the character of service rendered by this association, planted there by its founders, who were themselves pioneers and co-partners in bringing to American agriculture and its related interests the advantages of scientific investigation and experiment.

In his history of the Sheffield Scientific School of Yale University, Professor Chittenden says that Professor Norton "above all others deserves the title 'founder,' for upon him depended the very existence of the school in the critical years of its adolescence." Professor Johnson himself gave a generous measure of credit to Professor Norton for his influence in helping to establish the first experiment station in the United States. Of Professor Johnson one commentator has said, "He was a pioneer of pioneers, a leader of thought, the disciple of a new idea of science." Another concludes, "The whole system of agricultural experiment stations in this country may well be regarded as his monument." Dr. Jenkins, in his monograph, "A History of Connecticut Agriculture," says that Professor Johnson's writings probably had the widest influence on farming in America of any in the nineteenth century. It seems particularly appropriate that we should have in the records of our association somewhat of the story of the careers of these two men because of their interest in, and influence upon, that branch of science in which we are all engaged.

There was little serious consideration of science as a field for exclusive thought and endeavor, or as an instrument of progress to civilization, until about the beginning of the nineteenth century. Between 1800 and 1850, however, there were marked indications of a real appreciation of its possibilities, although even then there were some who looked with disfavor, not without some concern, upon attempts to inquire into the hidden laws of nature, and there were still others who doubted the educational worth of scientific study. In Europe there were outstanding scholars in astronomy, geology, and physics, and there were also chemists of note, among whom, more particularly interested in agricultural chemistry, were such men as Sir Humphry Davy in England, whose views on agricultural improvement through the application of chemistry were set forth in his "Elements of Agricultural Chemistry" (1813); Lavoisier, who facilitated chemical measurements by the introduction of the analytical balance and who was in many senses the founder of our modern knowledge of animal metabolism; Pasteur, whose distinguished career began (1849) toward the close of the period we are considering; Mulder of Holland, whose noteworthy studies of proteins (1840) were of particular interest to agriculture and to nutrition; and Wöhler and Liebig in Germany, the latter perhaps the outstanding figure in the scientific world in his generation. It was he who established the first chemical laboratory specially equipped

for chemical investigation, and to him came students from all over the world to receive training and inspiration.

There were, moreover, many technical schools for the study of chemistry and other sciences both in England and in many of the countries of Continental Europe.

The facilities for scientific study in the United States during this period were much more limited. The courses of study in the most advanced of our colleges of that time still emphasized the classics, while science, if recognized at all, received incidental and quite subordinate consideration. Enthusiasts for science appear to have been regarded as a radical element in the field of education, a group to be tolerated rather than encouraged. Philadelphia was perhaps the center of scientific thought and activity. Here was the American Philosophical Society, founded as early as 1744, the outgrowth of Benjamin Franklin's club, which was formed for the purpose of discussion and debate upon matters of morality, philosophy and politics; and here too was the University of Pennsylvania where Dr. Benjamin Rush, who has been called the father of chemistry in America, was professor of chemistry. When Benjamin Silliman the elder was appointed professor of chemistry at Yale in 1802, he went to Philadelphia to prepare himself for his new duties. He shortly went to Europe, however, as did all scholars of that time who wished to secure any advanced instruction.

This brief outline of the status of chemistry, and more particularly of agricultural chemistry, incomplete as it is, will perhaps serve as a background for a better appreciation of the fact that at this time in the United States the educational value of chemistry itself was not generally accepted, and that agricultural chemistry was a new idea. To establish the worth of applied chemistry our pioneers were forced to start almost at the beginning.

Among the students of chemistry here at this period there were a few who visioned its usefulness as an aid to the advancement of agriculture. Conspicuous among these was John Pitkin Norton who, with his colleague, the younger Benjamin Silliman, established the School of Applied Chemistry at Yale, afterwards to become the Sheffield Scientific School. He was born in Albany, New York, in 1822, but his family later moved to the ancestral home in Farmington, Connecticut. As a student young Norton showed little interest in classical studies, but he manifested "an original genius for natural science." It was decided that he should become a farmer like his father, but that no effort should be spared to insure his thorough education. To this end he spent his summers in practical farm work and his winters in an ambitious program of study, carried on at Albany, New York City and New Haven, which included French, mathematics, chemistry, mineralogy, natural philosophy, entomology, anatomy, law, drawing and music. This schedule appears to have occupied his time until 1844, when he went abroad and spent two years at the laboratory

of the Agricultural Chemical Association in Edinburgh. He soon demonstrated his ability as an investigator, and his comprehensive study of the oat plant, presented before the Highland Agricultural Society, received much commendation and was awarded the first prize of fifty sovereigns. Returning to New Haven, he was appointed to the faculty of Yale College in the capacity of professor of agricultural chemistry. In this appointment the experience and judgment of the elder Silliman no doubt played a significant part. Simultaneous with his appointment was that of the younger Silliman as professor of analytical chemistry, whereupon these two young men found themselves responsible for a new educational experiment and for the fate of the School of Applied Chemistry. Professor Norton had by this time abandoned the idea of becoming a practical farmer, and he returned to Europe to complete preparations for his new duties, spending nine months of hard study with Professor Mulder at Utrecht. It is said that his laboratory days were from 12 to 14 hours long and that in addition he burned much midnight oil in the study of German and Dutch. Returning to his official duties, he threw himself with characteristic energy and zeal into his new work. In addition to the immediate demands of teaching he lost no opportunity of tongue or pen to spread the fundamental doctrines of scientific agriculture to the public at large. He maintained close contacts with agricultural societies and always manifested a sympathetic interest in the problems of the farm. In 1850 he wrote "A Treatise on Scientific Agriculture," which was awarded a prize of one hundred dollars by the New York State Agricultural Society and which was published afterwards as a text book for schools. He also published several scientific papers, notable among which is one by the interesting title "Researches on the Protein Bodies of Peas and Almonds, and a Body of Somewhat Similar Nature Existing in Oats," published in the *Journal of Science*, 1848. It should be remarked too that he shared with his young colleague the financial burdens of this new school because no funds were forthcoming from the college, and the department resources were limited to the income from a small donation and the tuition fees of students. This revenue never met the expenses of the laboratory, and deficits were regularly made up from private funds of these two young men. It was a disappointment to Norton, and a blow to the school, when his colleague withdrew to accept a post in another State, but he assumed the entire burden, and carried on with tireless energy, so convinced was he of the ultimate success of his enterprise. He revised and enlarged the courses of study in his department and secured recognition of it by the corporation in their act establishing the degree of Bachelor of Philosophy, which was awarded for the first time in 1852 to a class of six men who had successfully completed the course which he had prescribed. However his active and promising career was soon to be terminated. He had seriously overtaxed his strength, not only by his years of strenuous preparation but by his arduous, though brief, period of service as a professor, and he died

in October, 1852. In five brief years he had established scientific study on an enduring basis in his university and had wielded a vast influence in the cause of chemistry as applied to agriculture. All of his books, manuscripts and apparatus he gave to the school, and in a memorandum written just before his death he expressed his ambition for his school in these words, "I hope it will be kept up, it has cost me a great deal of labor."

Before passing from this phase of our story, let us glance at the laboratory which constituted the department known by the rather imposing title "School of Applied Chemistry." We quote from a word picture of it by a student in Professor Norton's first graduating class:[1]

It was a plain wooden building, two stories high, painted white, which had been built in 1799, and used for nearly half a century as the president's dwelling. No outside additions were made for its new use, but its interior was fitted up so as to be reasonably convenient. The old kitchen served as a laboratory for students in qualitative chemical analysis, the sitting-room for the more advanced students; the front parlor was at first made into a lecture-room, but later, as the number of students increased, it too was turned into a working laboratory. The little library-room held the books of reference and also did duty as an office and recitation-room; the president's study became the "balance-room," and the pantries did duty as chemical reagent closets. The scullery became the place where obnoxious gases were made; a smith's bellows in the cellar supplied air for the only blast-lamp, which occupied a table in the middle of the room above, and the kitchen range heated a sand-bath. Modern spectroscopy was as yet unknown but successive classes of students made observations on the lithium flame, or tested for other colored flames, in the dark kitchen closet. The second story was mostly used as dormitories for assistants and students.

We pass now from the teacher to his distinguished pupil.

Like Professor Norton, Samuel W. Johnson also possessed a genius for natural science, and he was convinced of the tremendous benefits which would accrue to agriculture and its related interests through the application of chemical study and investigation. As a device for bringing this program about, he was particularly impressed with that type of institution which he had seen in effective operation abroad, the agricultural experiment station. His greatest work was done in the capacity of a missionary in educating the public to an appreciation of sound principles of agricultural science and the advantages of field experiments controlled and interpreted by scientific reasoning. For twenty years before his dream became a reality he had been doing precisely the sort of public service which his proposed experiment station was to render. His reports made to the Connecticut Agricultural Society and to its successor, the State Board of Agriculture, are in truth experiment station reports of the highest order.

Samuel Johnson was a native of New York. His parents lived first at Kingsboro and later at Deer River, and we find him at the age of sixteen teaching school, many of the pupils being nearly as old as their teacher.

[1] "History of the Sheffield Scientific School," by Russell H. Chittenden. Yale University Press, 1928.

He was at this time the proud possessor of a private laboratory, his father having set aside a small farm building for his use, equipped it with running water, and donated fifty dollars for the purchase of chemicals. Not to be outdone, his invalid mother contributed her wedding spoons to be converted into reagents. He had decided to pursue his chemical studies at Harvard, but a visit to New Haven and an interview with Professor Norton changed his plans, and in January, 1850, he went to Yale to study in the New School of Applied Chemistry. Here under the inspiring leadership of Professor Norton he made rapid progress and soon, on the advice of his teacher, decided upon a course of study abroad. His plans were interrupted in 1851, however, by his appointment as professor in the State Normal School at Albany, New York. Here he found his work pleasant and his surroundings congenial, but he was eager to carry out his plans for foreign study and he returned to New Haven in the fall of 1852 to complete his preparations. In May, 1853, he sailed for Europe and spent two years with such leaders in chemistry and agricultural science as Erdmann and Neumann in Leipzig, von Liebig, Pettenkofer and von Kobel in Munich, and Frankland, Lawes and Gilbert in England; and he also spent some time in Paris among French chemists. He returned home in August, 1855, whereupon he took up work in the newly organized Scientific School at Yale, an outgrowth of Professor Norton's School of Applied Chemistry, to the faculty of which he had received an appointment.

He was now largely occupied with teaching and investigation, but he found time for some public lectures and much writing. While yet a student in Germany he began a series of articles on agricultural topics which were sent home and which found publicity through the reports of agricultural societies and the agricultural press, and he also contributed with some regularity to the agricultural column in the *New York Tribune*. In addition to articles discussing results of his own investigations and observations, he wrote many critical reviews of current scientific literature. His paper presented to the Agricultural Society of New York at Albany in 1856 on "The Relations Which Exist Between Science and Agriculture" is of interest both because of its substance and because of the circumstances which prompted the effort. It was the hope of the more progressive members of that Society, among them Mr. Tucker, publisher of the *Country Gentleman*, Dr. Ezra S. Carr, chemist to the Society, and others, to found the "University of Albany," which it was proposed to make a center of scientific education, and to which it was hoped to attract leaders in agricultural science and other sciences. It was their purpose to have Professor Johnson join in this movement, entertaining the hope that his ambition for the founding of an experiment station might be realized sooner there than it seemed likely to be in Connecticut. The address attracted wide interest, but the hopes of these leaders were not realized.

A year later he read a paper before the Connecticut Agricultural So-

ciety on the subject of "Frauds in Commercial Fertilizers." This paper attracted wide attention, and no doubt it had a significant effect in furthering the cause which he was so actively sponsoring. In this report he cited the work of several of his contemporaries in this country and in Europe by way of supplementing his own observations. The idea of commercial valuations of fertilizer materials had been introduced by Soeckhardt in Germany, in 1849, and it was adopted at once by agricultural chemists elsewhere, notably in England and the United States. In calling attention to this feature, Professor Johnson introduced a convincing argument. It was difficult to impress farmers with the value of carefully planned investigations into the mysteries of plant nutrition because the prospects of practical results were too remote, but the idea of checking fraud and the thought of evaluating deficiencies in plant food in terms of money, with the further possibility of adjustments between buyer and seller on the basis of such valuation, provided a point of view which could not fail to be appreciated. It has been remarked frequently that the acceptance of the experiment station idea, involving the broad purpose of fundamental scientific investigation in agriculture, was hastened, if not entirely brought about, by this incidental detail in the larger picture.

Another important work was his series of lectures before the Smithsonian Institution of Washington, afterwards embodied in his book "How Crops Grow" which, with its later companion volume, "How Crops Feed," was widely used as a text, not only in this country but in many countries abroad. His paper on "Peat and Its Uses as Fertilizer and Fuel" gave a complete résumé of knowledge then available on that subject. A critic writing as late as 1910 commented that it contained substantially all that was known even at that time.

These activities were embraced in the period 1856 to 1870. It will be remembered that during that time (1860) the Federal Land Grant Act was passed. It provided for the donation of certain public lands to the States, the proceeds from the sale of which were to be used, with definite restrictions, for purposes of agricultural education. The institutions taking advantage of this law became known as Land Grant Colleges, or Colleges of Agriculture and Mechanic Arts. In Connecticut the Scientific School at Yale, now become the Sheffield Scientific School, was designated by the legislature as the institution to receive the funds derived from this source.

Events that soon took place crystallized public opinion on the question of experiment stations. The report on fertilizers which Professor Johnson made to the Board of Agriculture in 1869 attracted the attention of the Honorable Frederick Watts, United States Commissioner of Agriculture, who wrote for fuller details of the investigations made. A national convention was called by the Commissioner; it was held in Washington in 1872 and was attended by about 100 delegates, including leaders in agri-

cultural chemistry and scientific agriculture from various parts of the United States. This meeting attracted wide public interest to the plan of establishing a system of experiment stations as a matter of national policy. Meanwhile in Connecticut a bill was introduced in the General Assembly of 1874 providing for the establishment of an institution of this kind, the bill embodying provisions approved by the special committee of the Board of Agriculture of which Professor Johnson was chairman. A year later the station was established, first as a private enterprise under the control of Wesleyan University with Professor Atwater, one of Professor Johnson's students, in charge, and two years later as a State institution, at which time it was transferred to New Haven and Professor Johnson became director.

Thus inaugurated, the idea was adopted in other States, where similar institutions now rapidly appeared. This development brought profound satisfaction to Professor Johnson and his friends and colleagues, who by this time were a numerous group. To attempt to enumerate those who first and last lent support and encouragement to the cause would be at the risk of overlooking some who deserve mention, but among those whose names are conspicuous in reports and in private correspondence of Professor Johnson, are Silliman, Norton, Porter, Brush, Gillman and Brewer, colleagues of his at Yale; Dyer, Clift and Weld of the State Agricultural Society of Connecticut; Tucker and Carr of New York; Storer of Massachusetts; Goodale of Maine; Collier of Vermont; Cook of New Jersey; Pugh of Pennsylvania; and Higgins of Maryland.

Fertilizer laws had by this time been enacted in many States, and it is natural that fertilizer analysis should have become at first an important, or the chief, item of business in nearly all of these newly created institutions. However, the broader aspects of agricultural service did not fail to receive attention. In many of these stations nutrition problems and the study of the composition of feeding stuffs and of foods were undertaken at an early date.

Very soon the need for uniformity of ways and means of sampling and of analytical procedure became apparent. The initiative in this direction appears to have been taken by Commissioner Henderson of Georgia, who called representatives from the various experiment stations, from state departments of agriculture and from the government to a conference. A letter from Professor Johnson in reply to the Commissioner is of interest. It says in part:[1]

May 27, 1880

DEAR SIR,

Your proposal for a convention of agricultural chemists to agree upon methods of fertilizer analysis for common use, is in my opinion a very timely suggestion and I am entirely in favor of it. As to time and place of meeting, would it not be well to make them co-incident with some gathering at which many of those interested would

[1] Quoted from Elisabeth A. Osborne's book, "From the Letter Files of Samuel W. Johnson."

be likely to be present—say the annual meeting of the Am. Association for the Advancement of Science, held at Boston or Cambridge in August?

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Touching again upon this subject, a later communication from Professor Johnson to a colleague is also of interest to us and not without a touch of humor.[1]

May 8, 1884

MY DEAR SIR,

Prof. Brewer and Dr. Jenkins think I ought to go next week to Atlanta, Ga., to attend a convention called by the Commissioner of Agriculture of Georgia to discuss methods of determining "Reverted Phosphoric Acid." There is a very unhappy muddle in this matter already, and, with your approval, I will prepare to go and see what can be done to prevent things from getting more mixed.

Yours,
S. W. Johnson

And thus the idea of an association to devote itself exclusively to the study and formulation of analytical methods became another contributing factor in the plan for the advancement of agricultural science. Such an association, conceived of in 1880, became an established fact four years later when Wiley, Richardson, Gascoyne, Jenkins, and others founded this Association of Official Agricultural Chemists and chose their colleague, Professor Johnson, to be its first president. Dedicated at first to a rather restricted program of activity suggested by the immediate and pressing needs of the moment, its scope was soon broadened to include studies of methods for official and other investigations dealing with commodities and problems of general agricultural interest and importance.

The history of this organization has been admirably related on occasions similar to this in the past, and we need not dwell upon that now, except to say that the high purpose of the leaders in agricultural science, among whom are numbered the founders of this body, have been maintained in a continuous record of sound public service. When we recall that our association had its origin in the needs of its members for the better discharge of a common public service it seems fundamentally sound that we should adhere to this general principle in shaping our future policies and programs of work.

We can view the present status of our activities with real satisfaction. The scope of work contemplated by our staff of referees and associate referees was never more comprehensive than now, and we have just cause for gratification in the annual contributions of these workers upon whom our progress largely depends. The revision of our book of methods, now nearing completion, will bring that work to a state of excellence not heretofore attained. In this work many of us gladly cooperate, but the burden always falls upon a selected few. I wonder if we fully appreciate what this task involves in personal sacrifice. Our *Journal* by reason of contributed

[1] Quoted from Elizabeth A. Osborne's book, "From the Letter Files of Samuel W. Johnson."

papers and book reviews, has become of added interest and increased usefulness. And finally, the revision of those volumes of Dr. Wiley on "Principles and Practice of Agricultural Analysis" promises to be of unique value in the enlarged field which it covers. But I am sure that I read your thoughts correctly when I suggest that these accomplishments properly serve only as encouragements for continued effort and greater undertakings; they must not be allowed to create an attitude of complacency.

To increase the effectiveness of our work and to broaden its usefulness is the dominating thought in the minds of us all. To this end we may well give our individual thought to ways and means not only of increasing the interest of members who are now active in our work, but particularly of bringing into active participation many who are engaged in work in which agricultural chemistry plays a conspicuous part, and whose closer affiliation with us would be a benefit to them and to us. It will no doubt occur to many of us that there are such opportunities in our own institutions for thus enlarging representation in this body, thereby increasing the scope of its usefulness. The demands of research and control in the matter of analytical methods are not necessarily divergent and the procedure evolved for the one purpose often proves useful or suggestive for the other. For many years we have had in our official methods a procedure for the quantitative determination of boron, but so far as my observation goes it was so seldom used that it was hardly more than of academic interest. Quite unexpectedly, however, adaptations of it became of importance with the occurrence of boron in certain potash salts used for fertilizer purposes, and more recently boron determinations again assumed importance in connection with research in the nutrition of plants, notably tobacco. The determination of various forms of nitrogen are of interest alike to fertilizer control and to investigations in plant nutrition and the study of soils. The examples might be multiplied.

The question of a change in the program of our annual meetings arises at intervals and hinges upon the advisability of a series of sectional sessions. By some it will be felt that our membership is not large enough to warrant such a policy, and to others the disadvantages to members who are interested in two or more of our general subjects will be impressive. On the other hand we cannot deny the conspicuous success of the drug section, which for many years has been conducted in a separate session. We all recognize also the increasing amount of work devolving upon members of the fertilizer group, notably those engaged in formulating definitions. To be sure this latter topic is a committee activity, but it engages nearly all the members of the fertilizer section. Could the referee reports on fertilizers profitably be combined with this committee activity and assigned to a separate session with a suitable allotment of time for proper disposal of the business in hand? There are other subdivisions which would appear to be logical, but the plan should not be too much extended

until its advantages or disadvantages have been demonstrated. I offer this as a suggestion for careful consideration rather than as a recommendation, but I am impressed with the thought that greater interest and freedom in discussion is likely to be found in a relatively small group of members with a common interest than can be expected in a large group including many who are not particularly interested in the subject of the moment. At any rate, in an association like this where trial and experiment are a part of the daily program of us all, there would appear to be no serious objection to a judiciously planned experiment of this sort.

There is still another thought which impresses itself upon our minds as we close this hour. Death takes its inevitable toll, and the passing of such men as Power, Doolittle, Hortvet and Balcom remind us of how heavy this toll may be. And within the year we have sustained the loss of four other stalwarts in the councils of this association, Dr. Wiley, Dr. B. B. Ross, Dr. Randall and Dr. Read. It is to the glory of men and women like these that they cannot be replaced because they have established themselves in our hearts as well as in our work. We cherish the memories of them all, not alone for what they have done for us, but for what they have been to us. We can do them no greater honor than to follow their example; and we can serve our association in no greater measure than to develop worthy successors to these members who have been towers of strength among us and who typify the best in public service.

ORDER OF PUBLICATION

The reports of the committees presented on the last day of the annual meeting are given at the beginning of the proceedings, not in their chronological order. This arrangement will assist the referees, associate referees and collaborators in planning and developing their year's work. The remainder of the proceedings will then follow in the usual order.

THIRD DAY

WEDNESDAY—MORNING SESSION

REPORT OF REPRESENTATIVES AT NATIONAL CONFERENCE ON PHARMACEUTICAL RESEARCH

Your appointed representatives attended the annual meeting of the National Conference on Pharmaceutical Research, which was held at the Willard Hotel, this city, on May 12, 1930.

The usual custom of hearing reports from the 16 affiliated organizations was abolished, therefore no opportunity was given your delegates to present the report that they had prepared. Aside from the usual routine in the conduct of such a meeting, the various chairmen of the Standing Committees gave interesting reports.

S. L. Hilton for the Committee on Dispensing Pharmacy stated that several methods had been tried for coating enteric capsules. A successful method described the use of white shellac in spirit of ammonia as a first coating, to be followed by the usual coating of salol. Experimental tests showed that these coatings were resistant to weak hydrochloric acid solution and disintegrated in weak sodium hydroxide solution. When prepared by this method, these capsules have proved satisfactory to practicing physicians.

E. F. Cook sent out requests to the manufacturers of pharmaceuticals to submit suggestions for improvements in official preparations for the U. S. Pharmacopeia and the National Formulary, particularly those dealing with the keeping quality of the articles. A number of replies were received with suggestions for stabilizing these products.

W. L. Scoville reported for the Committee on Standardization of U. S. P. and N. F. Galenicals that stability was the most important question at present. Digitalis and ergot preparations are now receiving the most attention, but no satisfactory conclusions have been reached. It is hoped that the continued efforts will be successful.

The evaluation by chemical means of such drugs as gelsemium, lobelia, sanguinaria and veratrum viride have been attempted with indifferent success.

In the preparation of galenicals it appears that the menstruum for extracting a drug is an important factor in the stability of the preparation. Successful extraction consists in obtaining a menstruum that will not only remove the active principles but will also reject, as far as possible, the disturbing principles.

Antiseptics have received much attention by manufacturers according to D. E. Combs, who reported on the Manufacture of Medicinal Chemicals. New products have been developed with special reference to their bactericidal action. The decompositon of ether in tin containers is being overcome by improved processes of manufacture and by the use of some substance which will preserve the ether without contaminating it.

H. W. Youngken presented a full report on the Sources and Identification of Botanic Drugs. Specific mention was made of the work of the Insecticide Division of the Bureau of Chemistry and Soils on derris elliptica, from which rotenone has been extracted. Valuable researches on the microchemical detection of alkaloids in plants have shown that the various cinchona barks contain the four principal alkaloids in different proportions, with quinine and cinchonidine predominating. Reports from many sources indicate that there has been less adulteration of crude drugs on the American market during the past year than in former years. Lack of cleanness of the drug is the principal complaint. Various species of ephedra without alkaloid are used, and all are marked Ephedra Vulgaris.

B. V. Christensen for the Committee on Standardization of Botanic Drugs especially commended M. R. Thompson for his work on ergot. He stated that there is need for additional research on the microscopical characteristics of cascara amarga, on the biological test for capsicum, on the standards for black mustard, on the extractive yield of benzoin and on the biological and chemical assay of ergot.

A. R. Bliss suggested that collaborative investigations be started with reference to the bacteriology of fermentations observed in sirups, mucilages and other pharmaceutical preparations. Methods for the determination of vitamins A and D have been studied collaboratively by members of the American Drug Manufacturers Association, and a report has been submitted to the Association.

J. C. Munch for the Committee on Pharmacology and Bioassays recommended that standard samples of aconitine and ergotamine be prepared for distribution by the Food and Drug Administration in place of the usual liquid preparations. Pituitary extract should be assayed by a pressor diuretic method in addition to the U. S. P. oxytocic method. Other substances than ouabain as standards for digitalis and strophanthus should be investigated. It was recommended that the methods of assay for insulin adopted by the Toronto Insulin Committee be adopted by the U. S. P. Revision Committee.

The report of H. V. Arny on the Census of Pharmaceutical Research showed 553 workers engaged in various phases of pharmaceutical investigation.

H. R. WATKINS
L. E. WARREN

Approved

E. M. Bailey: There is one item that should have come after the Report of Representatives to the National Conference on Pharmaceutical Research, but it does not appear on this program. The association sent representatives to the U. S. Pharmacopeial convention, and Mr. Warren will make a brief comment on that so that it can be included in the report.

L. E. Warren: I have no formal report to make because I did not know until I came here today that the matter would be taken up at this time. However, for your information and for the record, I shall make a brief statement. The U. S. Pharmacopeial convention, as you probably know, meets in Washington once in 10 years. It met this year in May. The object of the convention is to elect a Committee of Revision, which in turn revises the U. S. Pharmacopeia. Also this convention elects a board of trustees and other officers to take care of the financial matters connected with the revision and the publication of the Pharmacopeia.

The Pharmacopeial convention is made up of delegates that are appointed by state and county medical associations, state and county pharmaceutical associations; certain governmental agencies, like the Department of Agriculture, the Department of the Treasury, etc.; and certain other organizations that are permitted by the convention itself to send delegates, and that are so named in the articles of incorporation. This association was represented by three delegates: the president, Dr. Bailey; the secretary, Dr. Skinner; and the speaker. While this association had but three delegates present, in a way it was represented by a number of our members who were present to represent other associations. For example, the Association of American Dairy, Food and Drug Officials was represented by Dr. LeMay of Texas, Mr. Frisbie of Washington, and the late Dr. Randall of Baltimore; also Dr. Dunbar, Dr. Mohler and Dr. Stockberger represented the Department of Agriculture; Dr. Wiley, Dr. Emery and Mr. Swenson represented schools of pharmacy, and Dr. Blanck, a medical school, and there may have been present other members of our association whose names I do not at this moment recall. The convention itself was composed of 401 delegates, representing these various associations and agencies. The Committee of Revision, also elected by the convention, consists of 50 members. The treasurer of the convention reported a balance on hand of over $110,000, which will be used, of course, in revising the Pharmacopeia; first, in carrying on certain research work

necessary to produce the evidence for admitting certain articles to or deleting others from the Pharmacopeia, and, second, to print the book. A portion of that money, $20,000, was set aside as a permanent research fund, the income only of which is to be used in research problems. So far as I can recall no member of this association was elected as a member of the Committee of Revision, but the association was honored by having one of its representatives elected secretary of the convention.

Approved

REPORT OF EDITORIAL COMMITTEE

As you know, at the meeting last year the editorial work of the association was coordinated into one general committee, composed of the boards of editors of our three publications, and you saw fit to make the secretary chairman of that committee.

The program provides that there be a specific report from each Board of Editors. The purpose of coordinating this work, of course, is to better facilitate the business management of the association. Later I shall read the treasurer's report in detail, but it is sufficient here, perhaps, to say that the editorial activities of this association now amount to about $10,000 per annum, so it is getting to be quite a business undertaking.

Dr. LeClerc has been asked to make a report on the detailed work of the revision of *Methods of Analysis*, which he will do, and you will hear from Mr. Deemer on the status of the *Journal*, so I shall not touch on those subjects. A detailed report was made by each Board to the Executive Committee, and the affairs of the association and its policies were very carefully discussed and determined. There will be some changes, some developments in the *Journal* and some in the *Book of Methods*, which will be reported to you by the several chairmen. Dr. Browne has asked me to make the report on *Principles and Practice*.

The work of revision has progressed. The page proof of Volume 2 is expected any day, and it is anticipated that copies of Volume 2 will be available early in the year. It will be a volume of about 750 pages. I believe it is going to be—or is already, because the detailed work on it is finished—a volume that this association is going to be greatly interested in, and one of which it will be very proud. The chapters were written by men who are recognized as leaders, and the book will have the prestige that a book of this kind should have. The delay in completing the work has been due to several causes, one of which was the action taken at the meeting last year to expand that part of the text devoted to standard foreign methods. Then the illness of one of the publishers further delayed us and made it difficult for us in getting out the cuts. Volume 3 was outlined for 38 chapters, and 25 chapters are in and mostly edited. It is anticipated that the remainder will be in very shortly, and we hope to have Volume 3 ready by January 1, 1932. Volume 3 is quite a problem;

typewritten manuscript now is over a thousand pages. It means that there will be printed pages with cuts amounting to approximately twelve hundred pages. It is impossible to consider this as one book, and yet we feel that we cannot very well reduce the subject matter of the several chapters. It will probably mean that Volume 3 will be printed in two parts, because our printers tell us that we cannot afford to produce a book of over 750 pages and expect to sell it for only $5.00. If we divide Volume 3 into two parts, it will mean, of course, that the set will cost approximately $20.00. Volume 3, like Volume 2, is outlined in our new scheme; each chapter has an author recognized in the field in which he is writing. Only such methods or parts of methods are given as to make the reading clear and understandable. The purpose of these books—and we have held to it quite definitely—is to supplement the *Book of Methods* and to provide the analyst with statistical and historical material and discussions of the significance of all determinations. It is truly a handbook for the interpretation of the analyst's findings. It has been a tremendous task, and I am frank to say that if Dr. Browne and I had understood and appreciated fully what it meant we should have hesitated to undertake the job. To attempt to coordinate and unify the work of at least 38 men and to get it into a sort of common language is a real task. For Volume 2 we have had splendid cooperation, and it has been a pleasure to work with these men. In passing I should like to say just a word or two about Dr. B. B. Ross, who produced the chapter on potash. It was one of the last things he did. I felt just a little cruel in urging him to finish that chapter, but it will now have for most of us an added importance and sentimental value. It will be recognized, I am sure, as an authoritative production. I should also at this time like to say a word in commendation of the work of two of my colleagues, Dr. W. H. Ross and Dr. Merz, without whose aid, I am frank to say, I do not think that Volume 2 would have been ready even by the first of January.

It is also planned, and it was discussed by the Executive Committee, to have Volume 1, on soils, revised. As you know, it was revised but slightly by Dr. Wiley, and it is not in harmony with Volumes 2 and 3, which, as has been emphasized, were entirely changed. Dr. Wiley consented to this change when I discussed it with him. While *Principles and Practice* will still remain a monument to Wiley, the books are brought down to date by men who are recognized as authorities in their several lines.

I believe that is all I have to report as General Chairman of the Editorial Committee, and we shall next hear from the subcommittees. Will Mr. Deemer please present the report of the editors of the *Journal*?

W. W. SKINNER

Approved

REPORT OF THE BOARD OF EDITORS

After receiving and considering proposals from several printing companies who specialize in the production of scientific and technical journals, the Board is in a position to report that the printing of the *Journal* can now be effected at a price that will enable the subscriptions to pay for the publication. However, we urge you to interest others in subscribing to the *Journal* and to support the publication of your association if you are not a subscriber. With the advantage gained by our price revision we should develop and maintain a lead in revenue.

There have been some requests for trimmed edges of the *Journal* and we have decided to accede to these demands. Beginning with the February number the new form will be adopted. We realize that there may be some of the members who may not consider this change desirable; we shall, therefore, be glad to hear from the members as to their wishes after they have tried the change out. The margin will permit of further trimming by those who wish to bind their *Journals*.

Although this year's subscriptions show only a slight increase over last year's, there have been several new subscriptions taken out, 6 in China, 5 in Japan and 1 in Portugal, a country in which we have never before had a subscriber.

Subscriptions, 1930:	
Domestic	599
Foreign	232
Total	831
Miscellaneous:	
Complimentary	13
Exchanges	17

R. B. DEEMER F. C. BLANCK
H. R. KRAYBILL W. S. FRISBIE
W. F. HAND

W. W. Skinner: Will Dr. LeClerc make the report of the Board of Editors of *Methods of Analysis*?

REPORT OF BOARD OF EDITORS, METHODS OF ANALYSIS

In accordance with the recommendation of the association, this committee has taken note of every action that has been passed on by the association during the past six years and has incorporated every change in the respective chapters. After those changes had been incorporated, the chapters were submitted to the general referee or associate referee, with the request that he go over the material, as thus modified, and make

further suggestions for improvement. He was asked to suggest the deletion of obsolete methods, and even to recommend the adoption of new methods.

As a result of all the suggestions and changes that have been made and adopted officially, almost 2,000 lines of obsolete material will be deleted, and in place thereof about 5,000 lines of new material will be inserted. It will appear from that statement as if the book will be 50 or 60 pages larger, but as a matter of fact it will not be, because we are going to use various abbreviations, for example: mg. for milligram; g. for gram; m.p. for melting point; soln. for solution; b.p. for boiling point; and temp. for temperature. We also intend to eliminate thousands of unnecessary words. For example, it is not necessary to say "dilute sulfuric acid (1+4)"; if it is 1+4, it is dilute, and the word "dilute" is unnecessary Common chemical terms, such as potassium iodide, potassium permanganate, sodium chloride, hydrochloric acid, etc., will be printed with the formulas instead of writing them out, and thus we shall be able to save more space. In going over this material the Committee on Revision has suggested that we insert headings for a number of chapters concerning which no material appears in the book. For example, there is nothing on bacteriological tests, marine products, nuts and nut products, radioactivity, fibers, paper and paper making, and vitamins. The headings for all these chapters will be inserted where they properly belong. The association has yet a great deal to do, and it is not going to stand still. The committee has also decided to include gelatine, which now appears in a separate chapter, in the chapter on meats and meat products, and vinegars in the chapter on spices and other condiments, thus eliminating two chapters. In the new book you will find approximately 150 more references than appeared in the old book. We shall have the copy ready for the printer as soon as possible.

Approved

W. W. Skinner: I should like to add a word or two, although the information will come out later in the report of the treasurer. In the account of the *Book of Methods* we have a balance of $2,803.50, and we have on hand 290 copies unsold. The demand runs about 50 copies per month. We think we have enough to carry us through until the period of the new issue, which will probably be, at the latest, June 1. I thought you would be interested to know that the financial condition, so far as the *Book of Methods* is concerned, is fairly satisfactory, but as Mr. Deemer has said, we are very much in hopes that the *Journal* itself can be made self-supporting.

Approved

No report was given by the Chairman of the Committee on Quartz Plate Standardization and Normal Weight.

REPORT OF THE COMMITTEE ON DEFINITIONS OF TERMS AND INTERPRETATION OF RESULTS ON FERTILIZERS

For Final Adoption as Official

1. ORDER OF TERMS

The *order of terms* in mixed fertilizers shall be nitrogen first, phosphoric acid second, and potash third.

2. STATEMENT OF GUARANTEES

The *statement of guarantees* of mixed fertilizers shall be given in whole numbers.

3. ACIDULATED FISH TANKAGE, ACIDULATED FISH SCRAP

Acidulated fish tankage, acidulated fish scrap, is the rendered product derived from fish and treated with sulfuric acid.

4. SIGNIFICANCE OF THE NAME OF A MATERIAL USED AS THE BRAND NAME OR PART OF THE BRAND NAME OF A MIXED FERTILIZER

When the name of a material is used as a part of the brand name of a mixed fertilizer, as for example blood, bone or fish, the nitrogen or phosphoric acid shall be derived from or supplied entirely by the material named. When the name of a material is used as a brand or as part of a brand and the nitrogen and phosphoric acid is not supplied by the material named, the word "brand" shall follow the name of the material. EXAMPLE: "Fish Brand Fertilizer."

5. AMMONIATED SUPERPHOSPHATE

Ammoniated superphosphate is a product containing superphosphate and/or dissolved bone and nitrogenous compounds, but without the addition of potash.

6. ACTIVATED SEWAGE PRODUCTS

Activated sewage products are made from sewage freed from grit and coarse solids and aerated after being inoculated with microorganisms. The resulting flocculated organic matter is withdrawn from the tanks, filtered with or without the aid of coagulants, dried, ground, and screened.

Second Recommendation as Tentative

1. PROCESS TANKAGES

Process tankages are the products made from crude inert nitrogenous materials by processing under steam pressure, with or without the use of acids, for the purpose of increasing the activity of the nitrogen.

These products shall be called "Process tankages" with or without further qualification.

The water-insoluble nitrogen in these products should test at least fifty per cent (50%) and eighty per cent (80%) active by the alkaline and neutral permanganate methods, respectively.

2. SHEEP MANURE WOOL WASTE

Sheep manure wool waste is the by-product from wool-carding establishments; it consists chiefly of sheep manure, seeds, and wool fiber.

3. AVAILABLE PHOSPHORIC ACID

Available phosphoric acid is the sum of the water-soluble and the citrate-soluble phosphoric acid.

4. PEAT

Peat is partly decayed vegetable matter of natural occurrence; it is composed chiefly of organic matter which has some nitrogen of low activity.

5. CHARRED PEAT

Charred peat is peat dried at such temperature as to cause partial decomposition.

6. SULFATE OF AMMONIA

Sulfate of ammonia is a commercial product composed chiefly of ammonium sulfate and containing twenty and five-tenths per cent (20.5%) or more of nitrogen.

7. CYANAMIDE AND UREA NITROGEN

Cyanamide and urea nitrogen shall be classified as synthetic non-proteid organic nitrogen.

8. DICALCIUM PHOSPHATE

Dicalcium phosphate is a manufactured product consisting chiefly of phosphoric acid in the dicalcic form.

9. HIGH CALCIUM LIME PRODUCTS

High calcium lime products are those classes of liming materials containing not less than forty-five per cent (45%) of calcium and magnesium oxides and not more than four per cent (4%) of their total oxides of calcium and magnesium as magnesium oxide.

10. HIGH MAGNESIUM LIME PRODUCTS

High magnesium lime products are those classes of liming materials containing not less than twenty-five per cent (25%) of their total oxides of calcium and magnesium as magnesium oxide.

11. QUICKLIME, BURNED LIME, CAUSTIC LIME, LUMP LIME, UNSLAKED LIME

Quicklime, burned lime, caustic lime, lump lime, unslaked lime, are liming materials having a high content of calcium oxide and magnesium oxide resulting from heating suitable carbonates until substantially all the carbon dioxide has been eliminated.

12. HYDRATED OR SLAKED LIME

Hydrated or slaked lime is the product obtained by treating quick lime with sufficient water or steam to combine with its oxides.

13. AIR-SLAKED LIME

Air-slaked lime is the product obtained by exposing caustic lime to the atmosphere, whereby it absorbs moisture and carbon dioxide.

14. GROUND LIMESTONE

Ground limestone is the product obtained by grinding calcitic or dolomitic limestone. Seventy-five per cent (75%) or more should pass a 100-mesh sieve, and it should contain not less than ninety per cent (90%) of calcium and magnesium carbonates equivalent to not less than forty-five per cent (45%) of the mixed oxides of calcium and magnesium.

15. GROUND SHELL LIME

Ground shell lime is the product obtained by grinding the shells of mollusks, seventy-five per cent (75%) or more of which should pass a 100-mesh sieve. It should contain not less than eighty per cent (80%) of calcium and magnesium carbonates, equivalent to not less than forty per cent (40%) of the mixed oxides of calcium and magnesium.

16. MARL, GROUND SHELL MARL

Marl, ground shell marl, is the product obtained by grinding natural deposits of shell marl. Seventy-five per cent (75%) or more of which should pass a 100-mesh sieve. It should contain not less than eighty per cent (80%) calcium and magnesium carbonates, equivalent to not less than forty per cent (40%) of the mixed oxides of calcium and magnesium.

17. WASTE LIME, BY-PRODUCT LIME

Waste Lime, by-product lime, is any industrial waste or by-product containing calcium or calcium and magnesium in forms that will neutralize acids. It may be designated by the prefixation of the name of the industry or process by which it is produced, i.e., gas-house lime, tanners' lime, acetylene lime waste, lime-kiln ashes, lime silicate, etc.

18. CALCIUM SULFATE, GYPSUM OR LAND PLASTER

Calcium sulfate, gypsum or land plaster is a product consisting chiefly of calcium sulfate. It is accompanied by varying quantities of impurities, and it contains about 20 per cent of combined water. It does not neutralize acids.

First Reading Tentative

1. AMMONIUM PHOSPHATE (FERTILIZER GRADE)

Ammonium phosphate (fertilizer grade) is a product resulting from a chemical union of mono-calcium phosphate with ammonia.

2. NET WEIGHTS

The weights appearing on packages of fertilizer, agricultural lime and liming material shall always mean net weight.

3. BASIC LIME PHOSPHATE

Basic lime phosphate is a superphosphate to which a sufficient quantity of lime has been added to insure in the cured product a substantial quantity of active lime

4. REVERTED PHOSPHORIC ACID

Reverted phosphoric acid is that part of the total phosphoric acid in a fertilizer that is insoluble in water but soluble in a solution of official neutral citrate of ammonia.

5. AGRICULTURAL LIME AND LIMING MATERIAL

Agricultural lime and liming material is any neutralizing substance containing calcium and magnesium oxides in condition and quantity suitable for use in agriculture.

6. LIME

The word *"lime"* when applied to liming materials means calcium and magnesium oxides.

Proposed for Future Consideration

1. ROCK PHOSPHATE
2. SOFT PHOSPHATE WITH COLLOIDAL CLAY
3. TRI-CALCIUM PHOSPHATE
4. PRECIPITATED PHOSPHATE
5. PRECIPITATED BONE

H. D. HASKINS G. S. FRAPS
C. H. JONES R. N. BRACKETT
J. W. KELLOGG

REPORT OF COMMITTEE ON REVISION OF METHODS OF SOIL ANALYSIS

For the first time in a great many years we have not had a quorum of this committee present. In the revision of *Methods of Analysis* that is now under way, a change has been made from the old method of having a committee charged with the arduous labor such as you know was undertaken by the committee under the leadership of our lamented friend, Dr.

Doolittle. Dr. LeClerc has correlated all the work of the association and its referees, and if that be done, I do not see that it would be necessary for this special committee to be continued, and to make that provision in harmony with the revision work that is being done, I would move that the work of the committee be discontinued.

Approved

At the request of the Committee on Definitions of Terms, under the chairmanship of Dr. Haskins, I am asked to make the following motion: Moved that the name of the Committee on Definitions of Terms and Interpretation of Results on Fertilizers be amended to read "Definitions of Terms and Interpretation of Results on Fertilizers and Liming Materials," and that the General Referee on Fertilizers and the General Referee on Soils and Liming Materials be made ex-officio members of this committee. The reasons for this change are so self-evident that they do not call for an explanation. At the time this committee was appointed, there was no chapter on liming materials in the *Book of Methods*, and that subject was therefore not included, but the committee now advises that it is in a position to define such materials.

The motion was made, seconded and carried.

W. H. MacIntire

Approved

REPORT OF COMMITTEE ON RECOMMENDATIONS OF REFEREES

The association is again indebted to the staff of referees and associate referees and to their collaborators for the large volume of excellent work submitted. It is gratifying to state that a large number of reports were in the hands of the committee considerably in advance of the meeting, which greatly facilitated the work of reviewing them.

In view of the work on revision of our *Book of Methods* now in progress, referees and associates were asked both by this committee and by the Revision Committee to review the particular subjects and suggest such deletions and changes as they felt should be made to make the new edition as complete and as up to date as possible. Many of such suggestions were submitted directly to the Revision Committee, and some were included in the regular reports of collaborative work. Reports of collaborative studies were reviewed by subcommittees A, B, and C as usual; and, in addition these subcommittees reviewed with Dr. LeClerc all recommendations that have been made for changes in methods other than those of an editorial nature. Each suggested change was considered separately and acted upon according to the rules that govern action upon recommendations of referees.

E. M. Bailey

Approved

W. W. Skinner: In this connection I think it is desirable to bring to your attention a matter which it is necessary to consider at a 5-year-revision period of the *Book of Methods*, and to ask for the usual action that has been granted at the time of previous revisions. I should like to call your attention to the fact that in its methods this association has a unique possession—something we ought to be proud of, something we ought to adhere to—the ancient landmarks that have been observed in its development. There is no other association in the world, so far as I know, that gives the kind of study to methods that this one does. It is not enough that a method be a good method; a method may be good and never get beyond the tentative stage of adoption in this association. A method must be before this association two years before it is adopted as official. That is our governing policy now, and it has been the governing policy during the 46 years the association has been in existence. Our methods must have legal standing, and there must not be any question in a court of law about the legality of any of our actions. There are certain minor things, however, that must be handled. The motion which I shall make will be directed to that end. Before doing so, however, I want to read you this provision. By-law No. 2 reads as follows:

> These by-laws or any portion of them may be suspended at any regular meeting of the association without previous notice, by a vote of three-fourths of the active members present.

The specific thing just now is By-law No. 7, which reads:

> No method shall be adopted as tentative nor shall a tentative method be amended until such method or amendment has been recommended for adoption by the appropriate referee and published in the proceedings of the association.

Anticipating that we shall have a three-fourths vote, I am going to move that the by-laws be suspended, and the proviso of No. 7, "to be published in the proceedings," be waived. These changes will be published in the proceedings, but they may appear after the *Book of Methods* is published. In this connection, this matter was discussed at the Executive Committee meeting and the following action was taken:

> It was moved and carried that the Revision Committee, *Methods of Analysis* be instructed to make such editorial changes as may be justified for the purpose of obtaining uniformity and clarity.

I make the motion that the action of the Executive Committee be affirmed.

Unanimously carried.

REPORT OF SUBCOMMITTEE A ON RECOMMENDATIONS OF REFEREES

By A. G. McCALL (Bureau of Chemistry and Soils, Washington, D. C.), *Chairman;* R. N. BRACKETT, *Acting Chairman* and H. H. HANSON

WATERS, BRINE AND SALT

It is recommended that the study of the quantitative determination of minute amounts of boric acid be continued.

Approved

TANNING MATERIALS AND LEATHERS

No report was submitted.

INSECTICIDES AND FUNGICIDES

It is recommended—

(1) That Method I[1] be adopted as an official method for the determination of mercury in organic mercurial seed disinfectants (first action).

Approved

(2) That Method II[2] (precipitation method), described by the referee, be adopted as an official method for the determination of mercury in organic mercurial seed disinfectants (first action).

Approved

(3) That Method III[3] (titration method) for the determination of mercury in organic seed disinfectants be further studied, special attention being given to the reaction of the solution at the time of titration.

Approved

FLUORINE COMPOUNDS

It is recommended—

(1) That the modified method presented in the report of the associate referee for the determination of fluorine in insecticides be adopted as a tentative method.

Approved

(2) That collaborative and experimental study of this method be conducted next year.

Approved

CAUSTIC POISONS

It is recommended—

(1) That Method I[4] (Chapin) be adopted as an official method for the determination of phenol (carbolic acid) in such products as cresol, saponified cresol solutions, coal tar dips, disinfectants, etc. (final action).

Approved

(2) That Method II[5] (Hamilton and Smith modification of Chapin method) be adopted as an official method for the determination of phenol

[1] *This Journal*, 13, 156 (1930).
[2] *Ibid.*, 157.
[3] *Ibid.*, 158.
[4] U. S. Dept. Agr. Bull. 1308 (1924); *This Journal*, 13, 49 (1930).
[5] *Ind. Eng. Chem., Anal. Ed.*, 1, 232 (1929); *This Journal*, 13, 49 (1930).

(carbolic acid) in the presence of methyl salicylate in such products as fly sprays, disinfectants, etc. (final action).

Approved

SOILS AND LIMING MATERIALS

REACTION VALUE OF SOILS

No report was submitted.

AGRICULTURAL LIMING MATERIALS

It is recommended that in the method for the determination of carbon dioxide a silver sulfate suspension in sulfuric acid (1+19) be used in the carbon dioxide absorption train to insure removal of any sulfuretted hydrogen that may be evolved.

Approved

LESS COMMON METALS IN SOILS

It is recommended—

(1) That further collaborative study be given to the methods proposed by the associate referee for the determination of copper, manganese and zinc in soils, and that consideration be given to the applicability of this procedure to the determination of arsenic, iron, titanium, nickel and cobalt.

Approved

(2) The committee repeats the recommendations made for the past two years relative to the determination of boron and fluorine in soils.

Approved

IODINE IN SOILS

It is recommended—

(1) That the electric distillation method for the determination of iodine in soils be studied collaboratively.

Approved

FEEDING STUFFS

It is recommended—

(1) That the method of preparation of solution[1] and determination of sugars[2] in feeding stuffs be adopted as official (first action).

Approved

(2) That the methods for the determination of hydrocyanic acid formed by the hydrolysis of glucoside bearing material be further studied.

Approved

(3) That the use of the method for the determination of dried buttermilk in feeding stuffs by the identification of the lactic acid bacilli[3] be discontinued.

Approved

[1] *Methods of Analysis, A.O.A.C.*, 1925, 118.
[2] *Ibid.*, 119.
[3] *This Journal*, 11, 36 (1928).

STOCK FEED ADULTERATION

It is recommended—

(1) That further study of the Sterling method for the determination of hoof meal in meat by-products be discontinued.

Approved

(2) That the study of the methods for the detection of traces of ferrous sulfate, copper sulfate, and potassium iodide be continued.

Approved

MINERAL MIXED FEEDS

It is recommended—

(1) That the method proposed by the associate referee for the determination of lime in mineral feeds[1] be adopted as a tentative method, and that additional collaborative work be done.

Approved

(2) That further collaborative work be done on the determination of iodine in mineral feeds by the Knapheide and Lamb method,[2] and by other methods proposed for consideration.

Approved

(3) That methods for the determination of iodine in organic mineral mixtures be studied.

Approved

DETERMINATION OF MOISTURE

It is recommended that the electric air oven method[3] for the determination of moisture in feeding stuffs that do not contain sugars be adopted as official (first action).

Approved

SUGARS AND SUGAR PRODUCTS

HONEY

No report was submitted.

MAPLE PRODUCTS

It is recommended—

(1) That the official method of preparation of sample (*Methods of Analysis, A.O.A.C.*, 1925, sec. 99, p. 202) be dropped (first action).

Approved

(2) That the method recommended by the associate referee for preparation of sample be adopted as tentative.

Approved

(3) That the following words be added to the official method for the determination of total ash (*Methods of Analysis, A. O. A. C.*, 1925, sec.

[1] *This Journal*, 10, 177 (1927).
[2] *J. Am. Chem. Soc.*, 50, 2121 (1928).
[3] *This Journal*, 13, 40 (1930).

106, p. 203): "taking precautions to guard against, or to correct for, absorption of water during weighing" (final action).

Approved

(4) That the following paragraph be inserted after sec. 109, p. 203, *Methods of Analysis:* Alkalinity of Total Ash—Official. Add the alkalinities of the soluble and the insoluble portions (final action).

Approved

(5) That the directions for the Canadian lead number be modified as follows:

Title.—Insert "(Fowler modification)" after "Number."

Sec. 112, No change except the correction adopted last year—viz. "500 cc." instead of "50 cc."

Sec. 113, Substitute the following:

Weigh the quantity of sirup containing 25 grams of dry matter, transfer to a 100 cc. flask, and make up to mark at 20°, or use the solution in which the conductivity value has been determined (sec. 114). Pipet 20 cc. into a large test tube, add 2 cc. of the standard basic lead acetate solution, cork, and allow to stand 2 hours.

Filter with suction on a 25 cc. tared Gooch, having an asbestos mat at least 3 mm. thick. When nearly all the liquid has run through, fill the crucible with cold water. Repeat to a total of four washings, taking care to prevent formation of fissures in the precipitate by keeping it covered with water and avoiding too great suction. Dry at 100°, weigh, and multiply the weight by 20.

Approved

(6) That in the directions for the determination of conductivity value (secs. 114, 115, in addition to the emendations made last year (viz. the omission of "multiply by 10^{-5}" in sec. 114, and of "multiply the result by 10^{5}" in sec. 115), "25 grams of dry matter" be substituted for "22 grams of dry matter" and "to 20°" be inserted after "cool."

Approved

(7) That the conductivity value method as so amended be adopted as official (first action).

Approved

(8) That further study be made of the method of preparation of sample and of variations in the refractometric measurements of analysts.

Approved

(9) That study of lead value methods be continued with a view (1) to securing greater uniformity in the reagent; (2) to deciding whether it would be of advantage to use a smaller proportion (1.0 cc. or 1.5 cc.) of reagent, the analyst having in mind both the range of values in genuine sirups and sugars and the rate of decrease of value with content of genuine in adulterated products; and (3) to deciding whether the Winton, as well as the modified Canadian method, should be retained.

Approved

STARCH CONVERSION PRODUCTS

No report was submitted as no associate referee was appointed.

DRYING, DENSIMETRIC, AND REFRACTOMETRIC METHODS

No report was submitted.

POLARISCOPIC METHODS

It is recommended—

(1) That the study of the effect of lead clarification on the determination of sucrose in cane products be continued and extended to products of the raw sugar factory.

Approved

(2) That the study of inversion procedures on beet products, interrupted some years ago, be taken up again, with special reference to those containing raffinose.

Approved

CHEMICAL METHODS FOR REDUCING SUGARS

It is recommended—

(1) That the reducing power of invert sugar by the Munson and Walker method be studied with a view to corroborating or revising the tables, and that further experiments be made to determine the reducing power of levulose.

Approved

(2) That the study of Nyns' selective method for levulose[1] be continued, and that the effect of aldohexoses and pentoses be determined.

Approved

(3) That the iodine method for aldose sugars be studied with particular reference to the modification devised by Slater and Acree.[2]

Approved

(4) That the Scales method be adopted as tentative.

Approved.

FERTILIZERS

PHOSPHORIC ACID

It is recommended—

(1) That the words "place 2 grams of the sample on a 9 cm. filter" in the official gravimetric method for the determination of water-soluble phosphoric acid (*Methods of Analysis, A.O.A.C.*, p. 4, sec. 11, line 1) be changed to read "place 1 gram of the sample on a 9 cm. filter."

Approved

(2) That the words "treat 2 grams of the phosphatic material" in the official method for the determination of citrate-insoluble phosphoric acid in non-acidulated samples (*Methods of Analysis, A.O.A.C.*, p. 5, sec. 14 (b), line 2) be changed to read "treat 1 gram of the phosphatic material."

Approved

[1] Bull. Assoc. école sup. brasserie, Louvain, 25, 63 (1925); *C. A.*, 19, 1236 (1925).
[2] Unpublished.

(3) That the words "at the expiration of exactly 30 minutes" in the official method for the determination of citrate-insoluble phosphoric acid in acidulated samples (*Methods of Analysis, A.O.A.C.*, p. 5, 14 (a), line 9) be changed to read "at the expiration of 1 hour."

Approved

(4) That the changes indicated in recommendations (1), (2), and (3) be adopted as official (first action).

Approved

(5) That the collaborative study of the availability of superphosphates that have been treated with ammonia or other alkaline material be continued.

Approved

(6) That under the word "Determination," sec. 10 (a), p. 3, *Methods of Analysis, A.O.A.C.*, the following clause be inserted "not applicable in the presence of sulfates" (final action).

Approved

(7) That the first line of sec. 9, p. 3, be revised to read as follows: "Treat 2 grams of the sample as directed under 6 (a), (b), (c), or (d), etc." (final action).

Approved

NITROGEN

It is recommended—

(1) That the Robertson method[1] for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide or urea be adopted as official, with the following addition to be incorporated in the proper place in the procedure: "Use 5 grams of ferrous sulfate instead of 2 grams if the total nitrogen was found to be over 5 per cent," and that the words "2 grams of magnesium oxide" be inserted instead of 5 grams as in *Methods of Analysis, A.O.A.C.*, 1925, sec. 33, p. 11, (first action).

Approved

(2) That an attempt be made to devise a practical method for control work in determining accurately ammoniacal nitrogen in the presence of urea and cyanamide.

Approved

(3) That the Devarda method (*Methods of Analysis, A.O.A.C.*, 1925, 12) for the determination of nitrates in nitrate salts be made official (first action).

Approved

NITROGEN ACTIVITY METHODS IN FERTILIZERS

It is recommended—

(1) That the changes published previously,[2] with the exception that the error in the quantity, 25 grams instead of 50 grams, be made in sec.

[1] *This Journal*, 13, 38 (1930).
[2] *Ibid.*, 39.

40, p. 12, and in the revision published in *This Journal*, 11, 33 (1928) and adopted finally in 1928, *This Journal*, 12, 33 (1929) (final action).

Approved

(2) That to sec. 42, as revised in *This Journal*, 11, 34 (1928), a third paragraph be added (final action). This paragraph has been published.[1]

Approved

(3) That work on the determination of nitrogen activity in fertilizers be discontinued.

Approved

POTASH

It is recommended—

(1) That the Fraps method be further studied, magnesium carbonate being used in place of calcium carbonate and the modification of removing the water-soluble phosphates by the aid of heat, as suggested by the author, being tried. The method is as follows:

Fraps Method for Potash in Mixed Fertilizers

Weigh out 2.425 grams of the fertilizer sample, place in a 250 cc. beaker, and add 2 grams of precipitated calcium carbonate free from water-soluble potash and 75 cc. of water. Mix well. Allow to stand in the cold for 3–4 hours, rotating the beaker occasionally. Transfer with hot water the contents of the beaker to a filter, receiving the filtrate into a 250 cc. graduated flask. Wash the material on the filter with successive portions of nearly boiling water until the volume of the washings is 200–250 cc. Cool to room temperature, make up to the mark, and draw off an aliquot into a porcelain dish. Place on a water bath and evaporate to dryness. Add 1 cc. of strong nitric acid and 4 cc. of strong HCl, and evaporate to dryness on a hot plate under a hood. Repeat the addition of HNO_3 and HCl as above and again evaporate to dryness. Add strong HCl and again evaporate to dryness to insure the removal of all HNO_3. Take up with hot water and add a few drops of HCl and the usual quantity of $PtCl_4$ solution (have an excess sufficient to color the alcohol solution). Evaporate to a moist residue but not to complete dryness. Remove from the water bath and cover with 10 cc. of acidulated alcohol (10 cc. of strong HCl to 100 cc. of 95 per cent alcohol). Allow to stand 1 hour, then filter into a Gooch crucible that has been thoroughly washed with hot water. The insoluble residue is transferred to the crucible with the acidulated alcohol and washed until the filtrate is colorless. Wash the residue six to eight times with 10 cc. portions of the 20 per cent ammonium chloride solution. Wash the residue with 80 per cent alcohol as in the official method, dry at 100°C. for 30 minutes, cool, and weigh. Remove the chloroplatinate from the Gooch by washing carefully with hot water, dry 2 hours, and weigh. For a 50 cc. aliquot, multiply the weight of the double salt by 40 to obtain the percentage of potassium oxide.

Approved

(2) That the use of magnesium oxide in the official method, as suggested by Bible,[2] for the removal of water-soluble phosphates be studied.

Approved

[1] *This Journal*, 13, 39 (1930).
[2] *Ibid.*, 8, 420 (1925).

HIGH ANALYSIS FERTILIZERS

It is recommended—

(1) That the subject of moisture variation be given further study with a view to correction of analysis for such variation and that a method be devised that is applicable to materials that decompose at a temperature of 100°C.

Approved

(2) That methods for sampling, for preparation of sample, and for securing a proper portion of the sample for analysis be studied.

Approved

PLANTS

It is recommended—

(1) That the methods for the determination of iron and aluminum in plants published in *This Journal*, 11, 203 (1928), except that the fusion is made with a mixture of sodium and potassium carbonates instead of acid potassium sulfate, be adopted as tentative methods.

Approved

(2) That the micro method for the determination of iron presented in the report of the referee, and the micro method for aluminum given in last year's report[1] be adopted as tentative methods, and that study be continued upon them with the object of making them official.

Approved

(3) That the method presented in the report of the referee and other methods for the determination of fluorine in plants be studied.

Approved

(4) That the micro method submitted by the referee for the determination of calcium and the micro method for the determination of phosphorus be adopted as tentative and collaboratively studied.

Approved

PREPARATION OF PLANT MATERIAL FOR ANALYSIS

It is recommended—

(1) That the effect on the various carbohydrates of length of time of storage of the samples when preserved in alcohol be studied by means of methods proposed by the associate referee.[2]

Approved

(2) That methods of preparation of a sample for forms of nitrogen be studied.

Approved

LESS COMMON METALS IN PLANTS

It is recommended—

(1) That methods for the determination of copper, manganese and zinc

[1] *This Journal*, 13, 221 (1930).
[2] *Ibid.*, 40.

in plants, which were made official (first action) last year,[1] be given further study.

Approved

(2) That further study be given to methods for the determination of iodine in soils, agricultural limestones, forage crops and foods.

Approved

(3) That the electric furnace method for the determination of iodine described in the report of the referee be studied collaboratively during the coming year.

Approved

TOTAL CHLORINE IN PLANTS

It is recommended that work on methods of determining total chlorine in plants be continued, in view of the fact that this determination on some material is still attended with considerable difficulty.

Approved

CARBOHYDRATES IN PLANTS

It is recommended—

(1) That the methods referred to in the report of the associate referee be adopted as tentative for plants without further study.

Approved

(2) That studies be continued upon these methods with the object of either adopting them as official or modifying them.

Approved

(3) That studies be made especially upon methods of clearing, the determination of sucrose by the invertase method and the determination of starch by the takadiastase method.

NOTE: The above methods refer to the determination of reducing sugars (*Methods of Analysis*, *A.O.A.C.*, 1925, p. 190, **34 (a)** and **(b)** and **35**); sucrose (*Ibid.*, p. 119, **20**); starch (*Ibid.*, 119, **22** and p. 120, **23**).

Approved

PAINTS, PAINT MATERIALS AND VARNISHES

It is recommended—

(1) That the Standard Methods of Routine Analysis of White Linseed Oil Paints, D215–29 of the American Society for Testing Materials be adopted as tentative methods and that these methods be printed as a separate chapter, entitled "Paints" in the edition of *Methods of Analysis* now being prepared.

Approved

(2) That the following method be studied with the object in view that, after adapting them to the needs of the Association, action be taken to recognize them at the next meeting of the Association: (a) routine analysis of white pigments, (b) specifications for raw linseed oil, (c) tentative

[1] *This Journal*, 12, 35 (1929); 13, 40 (1930).

specifications for boiled linseed oil, (d) sampling and testing turpentine, (e) tentative specifications for petroleum spirits, (f) testing oleo-resinous varnishes.

REPORT OF SUBCOMMITTEE B ON RECOMMENDATIONS OF REFEREES

By L. E. WARREN (Food and Drug Administration, Department of Agriculture, Washington, D. C.), *Chairman;*
H. C. LYTHGOE and A. G. MURRAY

SPECIFIC GRAVITY AND ALCOHOL

No collaborative work was reported.

It is recommended that the subject be continued.

Approved

NAVAL STORES

TURPENTINE AND ROSIN

The associate referee reports that there has appeared no need for modification of the existing methods of the association for examination of rosin or turpentine oil and that no cooperative work has been done within the association on either subject during the past year.

It is recommended that work on naval stores be continued with special reference to methods for the analysis of rosin.

Approved

DRUGS

CRUDE DRUGS

It is recommended that the subject be continued.

Approved

RADIOACTIVITY IN FOODS AND DRUGS

It is recommended that the former topic "Radioactivity in Drugs and Waters" be continued in its relationship to both foods and drugs.

Approved

EMODIN BEARING DRUGS

Two projects are under way under this topic, viz., cascara and aloin.

ALOIN

It is recommended that the subject be continued with special reference to Method II (acetylization method) submitted by the associate referee.

Approved

CASCARA

It is recommended that the subject be continued.

Approved

MERCURIALS

It is recommended—

(1) That the method for the determination of calomel in calomel oint-

ment submitted by the associate referee be adopted as tentative. (See *Methods of Analysis*, 1930.)

Approved

(2) That the method for the determination of mercuric oxide in mercuric oxide ointment be further studied.

Approved

MICROCHEMICAL METHODS FOR ALKALOIDS

It is recommended—

(1) That the microchemical tests for the identification of atropine and pilocarpine,[1] now tentative, be adopted as official (first action).

Approved

(2) That the microchemical test for the identification of ephedrine be made tentative. (See *Methods of Analysis*, 1930.)

Approved

(3) That the alkaloids aconitine, arecoline, physostigmine, and yohimbine be studied collaboratively next year.

Approved

TERPIN HYDRATE

It is recommended that the subject be further studied.

Approved

SANTONIN

It is recommended—

(1) That the method (gravimetric) submitted by the associate referee for the assay of santonin in mixtures and tablets be adopted as tentative. (See *Methods of Analysis*, 1930.)

Approved

(2) That a study of methods of assay of santonin in crude drugs be undertaken.

Approved

ETHER

It is recommended that the subject be continued.

Approved

BIOASSAY OF DRUGS

It is recommended—

(1) That the method recommended by the associate referee for the bioassay of fluidextract of ergot be adopted as tentative. (See *Methods of Analysis*, 1930.)

Approved

(2) That the cat-eye method for the assay of mydriatic drugs and myotic drugs, which was adopted as tentative in 1927,[2] be now adopted as official (first action).

Approved

[1] *This Journal*, 11, 354 (1928); 12, 284 (1929).
[2] *Ibid.*, 10, 383 (1927); 11, 53 (1928).

(3) That the study of this subject be closed for the present.

Approved

EPHEDRA

It is recommended—

(1) That the method for the assay of ephedra now tentative[1] be amended by deleting titration procedure No. 1 and that the amended method be adopted as official (first action).

Approved

(2) That the quantitative method for the determination of ephedrine in tablets[2] be amended by deleting the section beginning with "see discussion," and ending with "convenient for a control," and substituting therefore Procedure No. 2 as described in the report of the associate referee, and that the method so amended be made tentative. (See *Methods of Analysis*, 1930.)

Approved

(3) That the qualitative color test for the identification of ephedrine by means of copper sulfate[3] be adopted as tentative.

Approved

(4) That the melting point determination described previously[3] be adopted as tentative.

Approved

(5) That the method for the determination of ephedrine in inhalants described by the associate referee but with the omission of titration procedure No. 1, be adopted as tentative. (See *Methods of Analysis*, 1930.)

Approved

THYMOL

It is recommended that the subject be continued.

Approved

MENTHOL

It is recommended—

(1) That the method described by the associate referee last year[4] be adopted as tentative.

Approved

(2) That the topic be considered closed.

Approved

BROMIDES-CHLORIDES

It is recommended that since modern potentiometric methods were not found to be satisfactory and fairly satisfactory chemical means of separation are already at hand, the topic be considered closed.

Approved

[1] *This Journal*, 12, 291 (1929).
[2] *Ibid.*, 13, 329 (1930).
[3] *Ibid.*, 330; *Methods of Analysis*, *A.O.A.C.*, 1925, 163.
[4] *Ibid.*, 12, 300 (1929).

OIL OF CHENOPODIUM

It is recommended—

(1) That the Paget method, as studied during the last two years and essentially as described previously[1] be adopted as tentative. (See *Methods of Analysis*, 1930.)

Approved

(2) That the subject be considered closed.

Approved

SALICYLATES AND OTHER PHENOLS IN MIXTURES

It is recommended that no further attention be given to the subject at this time.

Approved

SMALL QUANTITIES OF IODIDES IN MIXTURES

It is recommended that the subject be continued.

Approved

BISMUTH COMPOUNDS IN TABLETS

It is recommended that the subject be further studied.

Approved

COLORIMETRIC METHODS FOR VITAMINS

The associate referee and his predecessor, E. M. Bailey, made a thorough study of the available literature on the chemical identification of vitamins. The conclusion is that the time is not ripe for attention to this subject by this association.

It is recommended that the subject be discontinued for the present.

Approved

PHENOLSULFONATES

It is recommended that the topic be continued.

Approved

SULFONAL AND TRIONAL

It is recommended that the subject be further studied with a suggestion that some means be included for identifying the extracts obtained by organic solvents.

Approved

EMETINE

It is recommended that the method for the determination of emetine hydrochloride described by the associate referee be adopted as tentative. (See *Methods of Analysis*, 1930.)

Approved

CHLOROFORM AND CARBON TETRACHLORIDE

It is recommended—

(1) That the method for the assay of chloroform in mixtures described

[1] *This Journal*, 13, 336 (1930).

by the associate referee be adopted as tentative. (See *Methods of Analysis*, 1930.)

Approved

(2) That the subject be closed for the present.

Approved

GUAIACOL

It is recommended that the subject be further studied.

Approved

CALCIUM LACTATE

As the associate referee studied the subject with a view to determining lactic acid in calcium lactate and found no satisfactory method, it is recommended that the topic be considered closed until such time as promising work has been recorded.

Approved

IODOFORM

It is recommended—

(1) That the method described by the associate referee be adopted as tentative. (See *Methods of Analysis*, 1930.)

Approved

(2) That the subject be further studied with a view to determining iodoform in ointments and in gauze.

Approved

BEERS, WINES AND DISTILLED LIQUORS

No collaborative work was reported.

It is recommended that the subject be continued.

Approved

REPORT OF SUBCOMMITTEE C ON RECOMMENDATIONS OF REFEREES

By H. A. LEPPER (U. S. Food and Drug Administration, Washington, D. C.), *Chairman;* J. O. CLARKE and C. D. HOWARD[1]

DAIRY PRODUCTS

MILK

It is recommended that the method presented by the Associate Referee in 1929[2] for the determination of visible dirt in milk be further studied and that the associate referee confer with other associations interested in this line of work whose methods are now uniform with those of this association, with a view to preserving the existing uniformity.

Approved

[1] Resigned. G. G. Frary was appointed to this position.

[2] *This Journal*, 13, 237 (1930).

BUTTER

It is recommended—

(1) That the method, "Directions for Sampling.—Official,"[1] be dropped (first action).

Approved

(2) That the procedure for sampling tub and print butter outlined by the referee in his report be adopted as official (first action).

Approved

(3) That the type of container described by the referee in his report be adopted as the official sample container for butter samples (first action).

Approved

(4) That the mechanical stirrer method given in the 1927 report[2] be adopted as a tentative method.

Approved

(5) That a study be made of the shaking modification of the present official method, with a view to determining the best conditions for the preparation of sample butter and describing these conditions in order to eliminate any uncertainties.

Approved

(6) That the tentative method for the determination of moisture, fat, and salt,[3] be further studied.

Approved

CHEESE

It is recommended—

(1) That methods for the determination of lactose and sucrose in cheese be further studied.

Approved

(2) That the tentative methods for the determination of tartaric and citric acids in cheese, as amended in 1928,[4] be studied collaboratively.

Approved

(3) That the referee consider the need for further work on the phosphorus pentoxide: calcium oxide ratios of process cheese and of methods for the detection of preservatives, coloring matters, emulsifying agents other than above referred to, or other added substances, and recommend what studies be continued or dropped.

Approved

MALTED MILK

It is recommended—

(1) That the microscopic method for the identification of malted milk[5]

[1] *Methods of Analysis, A.O.A. C.*, 1925, 275.
[2] *This Journal*, 11, 276 (1928).
[3] *Ibid.*, 39.
[4] *Ibid.*, 12, 44 (1929).
[5] *Ibid.*, 238.

be adopted as tentative and that further work on the study of this method be discontinued at this time.

Approved

(2) That methods for the determination of butter fat in malted milk be studied.

Approved

DRIED MILK

It is recommended—

(1) That in place of the tentative method for preparation of sample for malted milk,[1] adopted as tentative for dried milk,[2] the method given in the referee's report be adopted as tentative for preparation of sample of dried milk and further studied.

Approved

(2) That further study be made of the details of the tentative method for the determination of fat in dried milk, especially as regards weighing the sample directly into the extraction apparatus and the quantities of reagents used.

Approved

(3) That study be made of a method of sampling dried milks.

Approved

(4) That study be made of the distillation method for the determination of moisture in dried milk.

Approved

For revision of *Methods of Analysis* it is recommended that the official Roese-Gottlieb method for fat, sec. 16, p. 262, be amended by omitting the first weighing of the flask, line 7, and requiring the use of a bead in the final extraction of the fat in petroleum ether to confirm the purity of the fat and by inserting a warning against wiping the flask just before weighing (first action).

Approved

ICE CREAM

It is recommended that the Referee on Ice Cream confer with the General Referee on Dairy Products to formulate a program of studies on methods for the examination of ice cream and that such studies as appear desirable be begun.

Approved

MILK PROTEIN

It is recommended—

(1) That the filtering directions of the tentative method for casein,[3] as modified last year,[4] be again modified to read as follows: "Add 0.5 gram

[1] *Methods of Analysis, A.O.A. C.*, 1925, 275.
[2] *This Journal*, 10, 35 (1927).
[3] *Ibid.*, 261.
[4] *Ibid.*, 13, 42 (1930).

of filter-cel, shake thoroughly, and filter clear through a suitable folded filter paper."

Approved

(2) By the referee that the method be given further collaborative trial.

The committee approves this recommendation for collaborative study and recommends that the study include other methods for the determination of casein and methods for other milk proteins.

For revision of *Methods of Analysis*, it is recommended—

A. That Method II, Casein, sec. **9**, p. 260, official, be deleted (first action).

Approved

B. That Method II, Albumin, sec. **11**, p. 260, official, be deleted (first action).

Approved

QUALITATIVE TESTS

It is recommended that methods for the determination of gelatin in milk be studied, with special reference to evaporated milk.

Approved

COFFEES

It is recommended that the problem of determining caffeine in so-called decaffeinated coffees be further studied.

Approved

MEAT AND MEAT PRODUCTS

It is recommended—

(1) That in the paragraph "Moisture," of the tentative method for the determination of added water in sausage and similar meat products,[1] between the words "hours" and "or," line 7, there be inserted the words, "or at a temperature of approximately 125°C. (not lower than 120° nor higher than 130°) for approximately 2–3 hours," and that there be added to the sentence ending line 8, after the word "hours," the statement "or 1 hour respectively."

Approved

For revision of *Methods of Analysis* it is recommended—

A. That in place of the first line of the tentative Sörensen method for the determination of amino nitrogen, sec. **34**, p. 248, the following words be substituted: "To 20 cc. of the filtrate from **27**, neutralized to phenolphthalein with barium or sodium hydroxide or to 20 cc. of an equivalent extract of the."

B. That secs. **37** and **38**, p. 248, "Soluble Phosphorus in Blood, Brain, and Glandular Organs.—Tentative," be deleted.

This is an obsolete method no longer being used, and the Committee approves this recommendation.

Approved

[1] *This Journal*, **13**, 42 (1930).

C. That the method for the determination of nitrites, sec. 14, p. 240, be deleted in view of the later tentative method,[1] as the methods are essentially identical.

Approved

SEPARATION OF MEAT PROTEINS

It is recommended that study on the separation of meat proteins be continued.

Approved

FATS AND OILS

It is recommended—

(1) That the "cold test"[2] remain as a tentative method, and that no further study of this method be made at this time.

Approved

(2) That the combined Reichert-Meissl[3] and Polenski method be made official and substituted for the present separate methods under "soluble" and "insoluble volatile" acids, with the exception that the illustration on page 292 be retained (final action).

Approved

(3) That the Kirschner method for the determination of volatile acids,[4] using standard solutions of sodium, potassium or barium hydroxide for the titration as described in the previous report, be made official (final action).

Approved

(4) That methods for the determination of moisture and volatile matter, with particular reference to the hot plate method, be further studied.

Approved

(5) That methods for the determination of the hexabromide number of drying oils (ether insoluble) be studied.

Approved

For revision of *Methods of Analysis* it is recommended—

(A) That the official Leffman and Beam method, secs. 27 and 28, p. 291, be deleted (first action).

This method is obsolete, and the committee approves the recommendation.

Approved

(B) That in the revised Reichert-Meissl value reagent (c) be changed to "Standard 0.1 N sodium or potassium hydroxide solution" and in the determination that "5 cc." be changed to "6 cc."

Approved

(C) That on p. 291, sec. 30 be changed slightly as recommended by the referee.

Approved

[1] *This Journal*, 8, 696 (1925).
[2] *Ibid.*, 12, 46 (1929); 13, 69 (1930).
[3] *Methods of Analysis, A.O.A. C.*, 1925, 290.
[4] *This Journal*, 13, 44 (1930).

BAKING POWDERS AND BAKING CHEMICALS

It is recommended—

(1) By the referee that the direct method for the determination of available carbon dioxide, as reported by the referee, be studied collaboratively this coming year.

The Committee recommends that the study include other methods for carbon dioxide, with a view to limiting, insofar as practicable, the number of methods for this determination.

Recommendation of Committee approved.

(2) That further study be made of the tentative method for the determination of aluminum by precipitation with phenylhydrazine.

Approved

For revision of *Methods of Analysis* it is recommended—

A. By the referee that in the Knorr method, sec. 2, p. 301, directions be included providing for an additional acid and two additional potash bulbs.

Inasmuch as a study of all the methods for carbon dioxide is called for, the Committee does not recommend this change at this time.

Recommendation of Committee approved.

B. That the official qualitative test for the determination of aluminum in the presence of phosphates, sec. 22, p. 308, be deleted (first action), and the test given in the referee's report substituted as a tentative method.

The principles of the official test for aluminum have long been superseded in general qualitative analysis. The Committee approves the recommendation.

Approved

C. That the method of Reynolds, Ross, and Jacob,[1] for the determination of fluorides be substituted for the present tentative method of determining fluorides in baking powder, secs. 35–38, pp. 312–14, including the figure on p. 313.

Approved

EGGS AND EGG PRODUCTS

It is recommended—

(1) That the method for the determination of total phosphoric acid (P_2O_5) given in the associate referee's report be adopted as tentative and further studied with a view to official adoption.

Approved

(2) That the method for the determination of fat (acid hydrolysis) described in the report of the associate referee be studied in comparison with the present tentative method and that the more satisfactory method be studied collaboratively.

Approved

[1] *This Journal*, 11, 231 (1928).

(3) That special studies be made by the associate referee of the method for the determination of lipoids and lipoid phosphoric acid described in the report of the associate referee in comparison with the present tentative method and that the more satisfactory method be studied collaboratively.

Approved

(4) That Mitchell's method for the determination of reducing sugars and sucrose, described in the referee's report, be studied collaboratively, including egg samples containing known amounts of added sugars.

Approved

(5) That paragraph (b), "Apparatus," of the 98°C. vacuum-oven method for the determination of total solids,[1] be changed to read as follows: "(b) *Air-tight desiccator.*—Should contain a fresh, efficient desiccant"; and that the method be adopted as official (final action).

Approved

(6) That the tentative 112°–117°C. air-oven method for the determination of total solids[2] be dropped, and work on this method be discontinued.

Approved

(7) By the referee that the tentative method for the determination of ash in eggs[3] be dropped and an attempt be made to devise a satisfactory method for all types of eggs.

The Committee approves this recommendation for the dropping of this method, made official first action, last year, which intensive study has shown is not applicable to all types of eggs.

Approved

(8) That the study of the method for unsaponifiable matter be continued and that the study include methods based on the constituents of the unsaponifiable matter.

Approved

(9) By the referee that the title of the tentative method, "Water-soluble Protein Nitrogen Precipitable by 40 per cent Alcohol" be changed to "Albumin Nitrogen," and that the method be modified according to the details given in the referee's report and further studied in conjunction with the same methods for alimentary pastes and flour.

The committee approves the recommendation for the adoption of the revised method. While recognizing the present title to be lengthy, it is not believed that the title "Albumin Nitrogen" accurately describes the substances determined, and as the recommendation for further study of the modified method is approved it is believed that the present title

[1] *This Journal*, 9, 56 (1926); 13, 49 (1930).
[2] *Ibid.*, 57.
[3] *Ibid.*, 12, 55 (1929).

should be retained until results of such studies suggest a more appropriate title.

Recommendation of committee approved.

(10) That further work be done to perfect the method for acid-soluble phosphoric acid.

Approved

(11) That the method for the determination of chlorine described in the referee's report be adopted as tentative.

Approved

(12) That the tentative method for the determination of chlorine be studied in comparison with that of Ulex,[1] for the determination of added salt.

Approved

(13) That methods for the detection and determination of glycerol be studied.

Approved

(14) By the referee that the determination of sterols be studied.

This study has been approved under the recommendation for the study of unsaponifiable matter above.

Approved

(15) That methods for the detection of decomposition in dried egg albumin be studied.

Approved

(16) By the referee that methods for determining albumin nitrogen as an index of decomposition in liquid egg be studied.

The committee approves this recommendation and recommends further that study of methods for dextrose be continued.

Approved

(17) By the referee that a study be made of total bacterial count as an index of decomposition.

The committee withholds for future consideration the question of the study of bacteriological methods, awaiting the establishment of a general policy on bacteriological methods by the association.

Recommendation of committee approved.

For revision of *Methods of Analysis* it is recommended—

A. By the referee that the changes described in the referee's report for "Methods of Sampling and Preparation of Sample" be adopted.

The committee believes that with the paucity of information on the sampling of eggs no real advantage is to be derived by changing the

[1] *This Journal*, 8, 57 (1925).

methods until the results of a comprehensive study of the problem are available.

Recommendation of committee approved.

B. By the referee that the tentative method for the determination of fat (acid hydrolysis) be amended as given in the referee's report.

The committee does not believe a change in the method should be made at this time as it is provided in the recommendations that the method be further studied collaboratively.

Approved

PRESERVATIVES AND ARTIFICIAL SWEETENERS

It is recommended—

(1) That the Monier-Williams method and other methods for the estimation of added sulfurous acid and added sulfite in food products be studied and that the number of methods for this determination be limited to the least practical number.

Approved

(2) That Recommendations 2, 3, and 4 from last year[1] be repeated.

Approved

(3) That the method for the determination of formic acid[2] be studied to determine its applicability to sugars and sugar products.

Approved

COLORING MATTERS IN FOODS

It is recommended—

(1) That additional samples of mixtures of tartrazine and amaranth be submitted to collaborative study.

Approved

(2) That sample mixtures containing the recently adopted dyes in conjunction with other permitted colors be studied collaboratively to test methods of separation and identification.

Approved

(3) That work be undertaken to separate and estimate quantitatively the recently adopted dyes in the presence of other permitted colors.

Approved

For revision of *Methods of Analysis* it is recommended that the chapter on coloring matters in food, tentative, as amended by the referee in a report to the committee on revision of methods, be adopted.

Approved

METALS IN FOODS

It is recommended—

(1) That the present official Gutzeit method for the determination of arsenic[3] be dropped and that the revised Gutzeit method, as reported by the referee, be adopted as an official method (final action).

[1] *This Journal*, 13, 72 (1930).
[2] *Methods of Analysis*, *A.O.A. C.*, 1925, 137.
[3] *Ibid.*, 171.

The revised method is recommended as a result of the action of a committee appointed at a symposium on methods for the determination of arsenic. The procedure does not involve the application of new basic principles.

Approved by special action.

(2) That the "arsine distillation" method for the determination of arsenic be further studied.

Approved

(3) That the "bromate" method for the determination of arsenic be further studied.

Approved

(4) That methods for the determination of tin be further studied.

Approved

(5) That the present tentative method for the determination of copper[1] be dropped, and the volumetric method given in the associate referee's report for 1929[2] be adopted as tentative.

Approved

(6) That further study be made of methods for the estimation of boron.

Approved

(7) That further study be made of methods for the determination of lead, especially as applicable to the determination of this element in spray residue.

For revision of *Methods of Analysis* it is recommended—

A. That directions proposed by the referee to the Committee on Revision for (a) treatment of sample, and (b) decomposition of organic matter, (1) by ashing and (2) by moist combustion for heavy metals, be adopted as tentative.

This procedure obviates the necessity for the preparation of sample in the first paragraph of sec. 5, p. 173, which paragraph can be deleted.

Approved

FRUITS AND FRUIT PRODUCTS

It is recommended—

(1) That the study of the refractometric method for the determination of soluble solids be continued.

Approved

(2) That a study be begun on the effects of a definite hydrogen-ion concentration of the extraction medium on the alcohol precipitate, pectic acid and ash of fruit and fruit products.

Approved

[1] *Methods of Analysis, A.O.A.C.*, 1925, 175.
[2] *This Journal*, 13, 426 (1930).

(3) That the tentative method for the determination of tartaric acid, sec. **18**,[1] be dropped.

Approved

(4) That the method for the determination of tartaric acid published previously[2] be adopted as a tentative method.

Approved

(5) That the tentative method for the determination of citric acid, secs. **24** and **25**,[3] be dropped.

Approved

(6) That the method for the determination of citric acid published previously,[4] with alternative directions providing for drying in a stream of dry air, be adopted as a tentative method.

Approved

(7) That work on the determination of fruit acids be continued.

Approved

(8) That the investigation of methods for the determination of the major bases, as well as chlorine in fruit ashes, be continued.

Approved

(9) That the methods referred to in the referee's report for the determination of potash, manganese, calcium and magnesium be adopted as tentative.

Approved

For revision of *Methods of Analysis* it is recommended that the tentative methods for the determination of malic acid, secs. **19** to **23** inclusive,[5] be dropped.

Approved

CANNED VEGETABLES

It is recommended that the study of methods for the detection of spoilage in canned foods be continued.

Approved

For revision of *Methods of Analysis* it is recommended—

A. That the tentative method, "Physical Examination," sec. **1**, p. 219, be revised as reported by the referee to the Committee on Revision.

Approved

B. That the method for "Preparation of Sample—Official," sec. **2**, p. 219, be revised as reported by the referee to the Committee on Revision.

Approved

C. That the method for "Butyric Acid (qualitative test).—Tentative," sec. **25**, p. 222, be dropped, because it is obsolete.

Approved

[1] *Methods of Analysis, A.O.A.C.*, 1925, 213.
[2] *This Journal*, 13, 104 (1930).
[3] *Methods of Analysis, A.O.A.C.*, 1925, 215–16.
[4] *This Journal*, 13, 99 (1930).
[5] *Methods of Analysis, A.O.A.C.*, 1925, 213–15.

D. That the new directions for the micro-analysis of tomato pulp, etc., reported by the referee to the Committee on Revision, be substituted for secs. 27–30, tentative.

Approved

CEREAL FOODS

FLOUR

It is recommended—

(1) That the method of sampling flour (official first action)[1] be made official (final action) and that further collaborative work be discontinued for the present.

Approved

(2) That until the chief protein constituents of wheat flour are reliably identified and correlated with flour properties or characteristics, attempts to develop methods for the quantitative determination of glutenin be discontinued.

Approved

(3) That further collaborative work be carried on with the tentative method (Rask) of determining starch[2] and that it be compared with the more recent modification of the Hartmann and Hillig method. (See p. 114.)

Approved

(4) That the tentative Seidenberg method for determining chlorine in chlorine-bleached flour be again studied and compared with a modification thereof which consists of adding ammonium or sodium bicarbonate to the ether extract and that studies be made for the detection of benzoic acid in flours treated with organic peroxides containing benzoyl radical.

Approved

(5) That special (non-collaborative) studies be continued on the methods for the determination of unsaponifiable matter in flour, alimentary pastes and baked products, in conjunction with the same study on eggs.

Approved

(6) That the study of rapid methods of ashing flour, alimentary pastes, bread and other baked products be continued.

Approved

(7) That a comparative study be made of the colorimetric and quinhydrone-electrode methods of determining hydrogen-ion concentration in order to ascertain the applicability of the colorimetric method to cereal products.

Approved

(8) That studies be made of methods of estimating the diastatic value of flour.

Approved

[1] *This Journal*, 9, 39 (1926); 13, 73, Rec. (1) (1930).
[2] *Ibid.*, 11, 37 (1928).

(9) That comparative tests be made of foreign and domestic methods of chemical analysis used as a measure of determining the value of flour.

Approved

(10) By the referee that the present tentative method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol be dropped and that in its stead the proposed tentative method for "albumin nitrogen" be adopted.

The committee approves the recommendation for the substitution of the tentative method but does not recommend the change in title for the reasons given under a similar recommendation on eggs. Further study is recommended in conjunction with similar methods for eggs and baked cereal products.

Recommendation of committee approved.

(11) That methods for the determination of carbon dioxide in self-rising flours be studied.

Approved

(12) That studies of the losses of ash on fusion be discontinued at this time.

Approved

BAKED PRODUCTS

It is recommended—

(1) That a study be made of methods to determine milk solids in bread.

Approved

(2) That a study be made of methods to determine rye in bread.

Approved

(3) That the method of determining chlorides in baked products (official first action)[1] be studied for the purpose of making this method official.

Approved

(4) That the tentative standard baking test be continued as such.

Approved

(5) That the tentative method for the determination of moisture in bread[2] be made official (final action).

Approved

(6) That methods for the determination of moisture in cake be further studied.

Approved

(7) That the method for the determination of total solids in air-dried bread by heating at 130°C. for one hour[3] be further studied.

Approved

[1] *Methods of Analysis, A.O.A.C.*, 1925, 232; *This Journal*, 12, 40 (1929).
[2] *This Journal*, 9, 42 (1926); 12, 40 (1929).
[3] *Ibid.*, 9, 40 (1926).

(8) That for the purpose of making the usual chemical determinations in bread one-half of the loaf be taken as a sample (official first action).

Approved

(9) That the tentative method for determining fat in bread by acid hydrolysis[1] be made official (first action).

Approved

(10) That further study be made of the method to determine lipoids in baked products.

Approved

(11) That the method (official first action) for the determination of crude fiber in air-dried baked cereal products,[2] be further studied.

Approved

(12) That methods for the determination of lipoid phosphoric acid and total phosphoric acid be studied, the principles of those methods found satisfactory for eggs being adopted, if possible.

Approved

ALIMENTARY PASTES

It is recommended—

(1) That the tentative method (official first action) for the determination of moisture in alimentary pastes[3] be further studied.

Approved

(2) That the method for the determination of crude fiber (official first action)[4] be further studied.

Approved

(3) That the tentative method for the determination of fat by acid hydrolysis be further studied.

Approved

(4) That the tentative method for the determination of lipoids and lipoid phosphoric acid be studied collaboratively in comparison with the chloroform-alcohol extraction method suggested by Mitchell.

Approved

(5) That the present tentative method of collecting and preparing a sample of alimentary paste for analysis,[3] be dropped and that the method given in the associate referee's report be substituted in place thereof.

Approved

(6) That the last paragraph of the method for the determination of total solids and moisture in alimentary paste[3] (tentative), be deleted.

Approved

(7) That methods for the determination of total phosphoric acid (P_2O_5) be studied, the principles of the method for eggs being adopted if possible.

Approved

[1] *This Journal*, 6, 61 (1922).
[2] *Methods of Analysis, A.O.A.C.*, 1925, 225; *this Journal*, 12, 41 (1929).
[3] *Methods of Analysis, A.O.A.C.*, 1925, 225; *this Journal*, 12, 43 (1929).
[4] *This Journal*, 9, 43 (1926).

VINEGARS

It is recommended—

(1) That the directions for polarization submitted by the referee be substituted for the present directions.[1]

(2) That for the present work on polarization methods be discontinued.

Approved

(3) That the first line of the method for the determination of sulfates,[1] sec. 24, p. 329, be changed to read as follows, and the method so changed be adopted as official (first action): "To 100 cc. of the absolutely clear sample."

Approved

(4) That methods for the determination of total and soluble ash be further studied, with particular attention given to the use of sucrose or other substances for reducing the time of heating and to the temperature of ashing.

Approved

(5) That the methods for the determination of phosphoric acid be further studied in connection with the studies on ash.

Approved

(6) That the method for glycerol be further studied.

Approved

(7) That the official method for the determination of total solids be studied, especially with reference to its application to vinegars high in solids, such as malt vinegar.

Approved

For revision of *Methods of Analysis* it is recommended—

A. That the method "Alcohol Precipitate.—Tentative," sec. 26, p. 330, be dropped.

Approved

B. That the method for "Pentosans.—Official," sec. 27, p. 330, be dropped (first action).

Pentosans are no longer determined in vinegar analysis. The results obtained by this method have no interpretative basis in the absence of authentic data on vinegars, especially those made by the quick generator process now being used in vinegar manufacture.

Approved

FLAVORS AND NON-ALCOHOLIC BEVERAGES

It is recommended—

(1) That the official Kleber method be removed from its place under the heading "Lemon and Orange Oils—Citral" and placed under the heading "Lemon and Orange Oils—Total Aldehydes" (final action).

Approved

[1] *Methods of Analysis*, A.O.A.C., 1925, 329

(2) That more extensive collaborative work be done on the gravimetric method for the determination of total aldehydes in orange and lemon oils and / or extracts, described in last year's report of the referee, or modifications of it and that the search be continued for other methods that are applicable to both oils and extracts.

Approved

(3) That collaborative work be done on the application of the tentative polariscopic method for the determination of oils of lemon, orange and limes in vegetable and mineral oils to solutions of these essential oils in glycerol and the acetic esters of glycerol.

Approved

GELATIN

It is recommended—

(1) That the methods for the determination of copper and zinc given in the report of the referee be studied along with the general methods for these metals in foods for the purpose of adopting the ones found most suitable for the determination of these metals in gelatin.

Approved

(2) That the study of the preparation of samples be continued.

Approved

For revision of *Methods of Analysis* it is recommended—

A. That the tentative method for the determination of lead, sec. **8**, p. 256, be deleted.

Approved

B. That the alternative method for the determination of copper and zinc, sec. **10**, p. 256, be deleted.

Approved

C. That the diffusion method for the determination of sulfur dioxide, sec. **13**, p. 257, be deleted.

Approved

CACAO PRODUCTS

It is recommended—

(1) That the modified method for the determination of crude fiber in bitter and sweet chocolates described in the report of the associate referee be adopted as official (first action).

Approved

(2) That the study of a method for the determination of crude fiber in milk chocolate be continued.

Approved

(3) That the method for the determination of milk proteins in milk chocolate be studied.

Approved

(4) That the study of the determination of foreign fats in cacao butter be continued.

Approved

(5) That the chemical method for the determination of lactose and sucrose in milk chocolate in which interfering non-sugar reducing substances are removed with an excess of mercuric nitrate in the presence of a slight excess of sodium bicarbonate be studied.

Approved

(6) That the method for the determination of moisture given in the referee's report be adopted as a tentative method.

Approved

For revision of *Methods of Analysis* it is recommended—

A. That the third paragraph under sec. 19, "Determination," p. 346, be changed as follows:

Insert the phrase "using 5 grams of fat" at the end of the first sentence, line 3;

B. Delete "by more than 2 degrees" from the sixth line;

C. Insert between the words "butter" and "adulteration" in the sixth line "by more than 3 degrees in the case of fat from chocolate liquors or sweet chocolate and by more than 6 degrees in the case of fat from milk chocolate."

Approved

D. That the heading "Reichert-Meissl Number.—Official" and the heading "Polenske Number.—Official," p. 347, be changed to read "Reichert-Meissl and Polenske Values.—Official," and appropriate reference change be made in the details of the method in the chapter on fats and oils.

Approved

SPICES AND OTHER CONDIMENTS

It is recommended—

(1) By the referee that the study of lecithin phosphoric acid determination in salad dressings be continued.

Because of the uncertainty of the stability of lecithin in frozen and dried eggs under some conditions other than evident spoilage, the committee recommends that the study of methods for estimating the egg content of salad dressings be not restricted to lecithin phosphoric acid.

Approved

(2) That methods for the determination of starch and sugars in prepared mustard be studied next year.

Approved

(3) That the method proposed last year by the referee,[1] for the deter-

[1] *This Journal*, 13, 480 (1930).

mination of reducing sugars before inversion in salad dressings be studied collaboratively along with the present tentative methods for the determination of total solids, oil, reducing sugars after inversion and total acid.

Approved

(4) That the method submitted by Mitchell and Alfend, slightly modified for the determination of the iodine number of paprika oil[1], be adopted as tentative and replace 18, p. 317, *Methods of Analysis*.

Approved

CHANGES IN THE OFFICIAL AND TENTATIVE METHODS OF ANALYSIS MADE AT THE FORTY-SIXTH ANNUAL CONVENTION, OCTOBER 20–22, 1930[2]

I. FERTILIZERS

(1) Under the word "Determination," sec. 10 (a), p. 3, the following sentence was inserted: "Not applicable in the presence of sulfates" (final action).

(2) The first line of sec. 9, p. 3, was revised to read as follows: "Treat 2 grams of the sample as directed under 6 (a), (b), (c), or (d), etc." (final action).

(NOTE: In last year's report an error was made in including "or (g)" in the preceding recommendation because sec. (g) had been eliminated by previous action.)

(3) In the official gravimetric method for the determination of water-soluble phosphoric acid, p. 4, sec. 11, line 1, the words "place 2 grams of the sample on a 9 cm. filter," were changed to read "place 1 gram of the sample on a 9 cm. filter" (first action).

(4) In the official method for the determination of citrate-insoluble phosphoric acid in acidulated samples, p. 5, sec. 14 (a), line 9, the words "at the expiration of exactly 30 minutes" were changed to read "at the expiration of 1 hour" (first action).

(5) In the official method for the determination of citrate-soluble phosphoric acid in non-acidulated samples, p. 5, sec. 14 (b), line 2, the words "treat 2 grams of the phosphatic material" were changed to read "treat 1 gram of the phosphatic material" (first action).

(6) The Robertson method, further[3] modified by inserting after the words "ferrous sulfate" the phrase "(if the total nitrogen is over 5 per cent, use 5 grams of ferrous sulfate)" and after "appear," the words "(If severe bumping occurs, add 10–15 glass beads)" was adopted as official (first action).

[1] *Ibid.*, 11, 524 (1928).

[2] Compiled by Marian E. Lapp, Associate Editor. Unless otherwise stated, all references in this report are to *Methods of Analysis*, *A.O.A.C.*, 1925.

[3] *This Journal*, 13, 38 (1930).

(7) The Devarda method for the determination of nitrates in nitrate salts, p. 12, was made official (first action).

(8) Further changes were made in sec. **40**, p. 12 and in the revised methods published previously[1] (final action). (See *Methods of Analysis*, 1930.)

(9) A third paragraph was added to sec. **42**, p. 13, and as revised, *This Journal*, **11**, 34 (final action). This paragraph has been published.[2]

(10) Page 1, sec. **1**, line 5, the words "Unless this process, etc.," were deleted.

(11) Sec. **10** was rewritten for the sake of clarity (first action). (See *Methods of Analysis*, 1930.)

(12) Page 39 **(a)**, line 7,[4] "25 grams" was changed to "50 grams" and after the word "liter," the words "protect from light and" were added.

II. SOILS

(1) Pages 21 and 22, secs. **5** and **6**, *Method II* was deleted, but the illustration (Fig. 2) was retained.

(2) Page 23, Fig. 4 and all of section **7 (b)** were deleted.

(3) The method for the determination of carbonate carbon was changed as suggested by the referee. (See *Methods of Analysis*, 1930.)

(4) Page 25, sec. **10**, par. 2, line 4, after the words "to settle" the words "4 hours" were inserted, and in line 7 the words "or filter . . . as directed above" were deleted.

(5) Page 27, sec. **13**, line 1, the words "or flat-bottomed" were deleted.

(6) The method for the determination of sulfur, p. 30, sec. **25**, was changed as suggested by the referee. (See *Methods of Analysis*, 1930.)

III. AGRICULTURAL LIMING MATERIALS

(1) In the method for the determination of carbon dioxide the use of silver sulfate suspension in sulfuric acid (1+19) was recommended in the carbon dioxide absorption train to insure removal of any sulfuretted hydrogen that may be evolved. (See secs. **7** and **8**, Soils, *Methods of Analysis*, 1930.)

(2) Page 36, sec. **7**, the words "2 grams of ground limestone or marl" were changed to "1 gram, etc."

IV. PLANTS

(1) The method for the determination of iron and aluminum in plants[3] published previously was adopted as a tentative method.

(2) The microchemical method submitted by the referee for the determination of iron only in plants was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

[1] *This Journal*, **11**, 33 (1928); **12**, 33 (1929); **13**, 39 (1930).
[2] *Ibid.*, **13**, 39 (1930).
[3] *Ibid.*, **11**, 203 (1928); **13**, 221 (1930).

(3) The microchemical method submitted by the referee for the determination of aluminum in plants[1] was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(4) The tentative method for the determination of calcium, p. 41, sec. **6**, was made official (first action).

(5) The microchemical method submitted by the referee for the determination of calcium was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(6) The tentative method for the determination of magnesium, p. 47, sec. **7**, was made official (first action) with the following modification: Change "25 cc." to "40 cc.," line 1, and eliminate the words "heat on hot plate or sand bath," line 2.

(7) The microchemical method submitted by the referee for the determination of phosphorus was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(8) The following methods recommended by the associate referees were adopted as tentative: reducing sugars, sucrose, starch, moisture, ash, ether extract, crude fiber and arsenic.

(9) In the method for the determination of sand and silica, p. 39, sec. **2**, line 1, the words "10–20 grams" were changed to "10–50 grams" (first action).

(10) The methods for the determination of manganese, calcium and magnesium, p. 40, secs. **4** and **5**, were dropped (first action).

(11) In the tentative method for the determination of copper[2] the word "manganese" in the last sentence was deleted.

(12) The sulfur-magnesium nitrate method, p. 45, sec. **18**, was made official (first action) with the following modifications: Change the words "250 cc. low-form Pyrex beaker" to "porcelain or sillimanite crucible."

(13) The tentative method for the determination of manganese[3] was dropped.

(14) The tentative method for the determination of phosphorus, p. 45, sec. **20**, was made official (first action).

(15) The official method for the determination of ferric and aluminic oxides, p. 39, sec. **3** was dropped (first action).

V. INSECTICIDES AND FUNGICIDES

(1) Method I[4] was adopted as an official method for the determination of mercury in organic mercurial seed disinfectants (first action).

(2) Method II[5] (precipitation method) was adopted as an official method for the determination of mercury in organic mercurial seed disinfectants (first action).

[1] *J. Am. Chem. Soc.*, **51**, 2730 (1929); *This Journal*, **13**, 221 (1930).
[2] *This Journal*, **12**, 35 (1929).
[3] *Ibid.*, 36.
[4] *Ibid.*, **13**, 156 (1930).
[5] *Ibid.*, 157.

NOTE: *In the previous publication Methods I and II were given as one method.* Separation has now been made for the sake of clarity.

(3) The modified method submitted by the referee for the determination of fluorine was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(4) In sec. **34**, line 5, the words "by means of 100 cc. etc.," were deleted.

(5) In sec. **56 (a)** the word "iodine" was changed to "bromate."

VI. TANNING MATERIALS

(1) In sec. **6**, line 5, "35 grams" was changed to "10 grams," and in line 18, "71 per cent" was changed to "72 per cent."

(2) After sec. **8**, the methods submitted by the referee for the determination of reducing sugars, total sugars, and non-reducing sugars[1] were inserted.

(3) Reagent **(b)**, sec. **15** was changed according to the recommendation of the referee. (See *Methods of Analysis*, 1930.)

(4) In sec. **16**, line 1, "25 cc." was changed to "50 cc."

VII. LEATHERS

(1) The method for the determination of moisture, p. 79, sec. **2**, dropped at the 1926 meeting,[2] was restored, with the following modification in the first sentence: "Plate 8–10 grams of the sample, as prepared under **1**, in a tared, wide, shallow weighing bottle (or a similar dish that can be covered tightly) and dry in an electric oven for 15 hours at 100°–102°C."

(2) The first line of the method for the determination of petroleum ether extract, p. 79, sec. **5**, was changed to read: "Place 5 grams of the leather, as prepared under **1**, in a fat-free thimble, cover with a layer of fat-free cotton, and extract in a Johnson or Soxhlet extractor with petroleum ether for 8–10 hours, distilling between 50° and 80°C." In line 7 the words, "periods of 1 hour," were changed to "periods of ½ hour." In line 9 after the word "volatilization" the words "or oxidation" were added.

(3) In the method for the determination of glucose, p. 80, sec. **9**, the words "filter at once through" were changed to "shake frequently for 3–5 minutes until all the phosphate has dissolved; then filter through, etc."

VIII. WATERS, BRINE, AND SALT

(1) On p. 83, sec. **6**, line 4, "105°C." was changed to "100°C." (first action).

(2) In sec. **7**, line 4, "105°C." was changed to "100°C." (first action).

(3) In sec. **8**, line 3, "105°C." was changed to "100°C." (first action).

(4) In sec. **10 (d)** the following directions were substituted (first action):

[1] *J. Am. Leather Chem. Assoc.* **23**, 105 (1928).
[2] *This Journal*, **10**, 3 (1927).

Dissolve 143 grams of sodium hydroxide in 950 cc. of water and filter through asbestos. Add 60 grams of red mercuric iodide to the filtrate and dilute with water to 1 liter. Mix thoroughly, allow to settle, and use the supernatant liquid.

(5) In sec. **33**, line 10, "50" was changed to "30" and "(1+5)" was changed to "(1+3)."

(6) On pp. 91–2, sec. **41**, line 7, "(1+5)" was changed to "(1+3)" and "dilute" and "(1+10)" in the 9th line were deleted.

(7) On p. 93, sec. **53**, line 2, the words "potassium hydrogen sulfate" were changed to "hydrochloric acid" (first action).

(8) On p. 94, sec. **54**, par. 2, "15" in the 4th line was changed to "10"; "dilute" was changed to "strong" and "(1+1)" was deleted in the 5th line; "−10" in the 8th line was deleted; and, "The dilute" was changed to "strong" in the 9th line (first action).

(9) On p. 96, sec. **61**, the phrase "with dilute ammonium hydroxide (1+10)" was inserted after "chlorides" in the 11th line.

(10) On p. 97, sec. **63**, the subheading "Amyl Alcohol Method—Official" was changed to "Ether-Alcohol Method—Official," and on p. 98, sec. **65**, the heading "Ether-Alcohol Method—Official" was changed to "Determination" (first action).

(11) On pp. 97–8 all of sec. **64** was deleted (first action).

(12) On pp. 104–6, sec. **83**, the words "The equivalent weights . . . are given in Table I" (last lines on p. 104) were deleted; all of "Table I" (p. 105) was deleted; the following words "For example, the reacting . . . $Ca(HCO_3)_2$, 20.487" (lines 15–26, par. 2, p. 106) were deleted; and the word "figures" (line 26, par. 2, p. 106) was changed to "values."

IX. FEEDING STUFFS

(1) The following methods, now tentative, were made official (first action): Preparation of solution, p. 118, sec. **18**; reducing sugars, p. 119, sec. **19**; and sucrose, p. 119, sec. **20**.

(2) The method proposed by the associate referee[1] for the determination of lime in mineral feeds was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(3) The electric air-oven method for the determination of moisture in feeding stuffs that do *not* contain sugars[2] was adopted as official (first action).

(4) Secs. **5** and **6**, p. 116, were deleted.

(5) In sec. **21**, line 1, after the words "this method" the words "is intended only for such materials as raw starch, potatoes, etc., and" were inserted.

(6) The tentative method for the detection of dried buttermilk[3] was dropped.

[1] *This Journal*, 10, 177 (1927).
[2] *Ibid.*, 13, 40 (1930).
[3] *Ibid.*, 11, 36 (1928).

X. PRESERVATIVES AND ARTIFICIAL SWEETENERS

(1) In sec. 3, p. 126, line 5, "10 per cent" was changed to "1 per cent" (first action).

XI. COLORING MATTERS IN FOODS

(1) The revision work on this chapter, tentative, submitted by the referee, was accepted. (See *Methods of Analysis*, 1930.)

XII. METALS IN FOODS

(1) The present Gutzeit method for the determination of arsenic, secs. 1, 2, 3, 4, pp. 171–74, was dropped. The method that was substituted and adopted as official by special action does not involve the application of new basic principles. (See *Methods of Analysis*, 1930.)

(2) The present tentative method for the determination of copper, sec. 8, p. 175, was dropped, and the method presented by the associate referee in 1929[1] was adopted as tentative.

(3) Directions proposed by the referee to the Committee on Revision for treatment of sample and decomposition of organic matter by ashing and by moist combustion were accepted. This action automatically deletes the first paragraph of sec. 5. (See *Methods of Analysis*, 1930.)

(4) Methods proposed by the referee for the determination of lead, fluorine, and manganese were adopted as tentative. (See *Methods of Analysis*, 1930.)

XIII. SUGARS AND SUGAR PRODUCTS

(1) The official method for the preparation of maple products (p. 202, sec. 99) was deleted (first action).

(2) The method proposed by the associate referee for the preparation of maple products was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(3) The words "taking precautions to guard against, or to correct for, absorption of water during weighing" were added to the official method for the determination of total ash, p. 203, sec. 106 (final action).

(4) The following paragraph was inserted after sec. 109, p. 203 (final action):

> Add the alkalinities of the soluble and the insoluble portions, 108 and 109.

(5) The directions for the Canadian lead number were modified as suggested by the referee. (See *Methods of Analysis*, 1930.)

(6) In sec. 115, line 1, the words "25 grams of dry matter" were substituted for "22 grams of dry matter" and in line 2, the phrase "to 20°" was inserted after the word "cool."

(7) As amended in (6) the conductivity value method was adopted as official (first action).

[1] *This Journal*, 13, 426 (1930).

(8) Under 6, "By means of a pycnometer," "(c) Tentative" was added as recommended by the referee. (See *Methods of Analysis*, 1930.)

(9) Under 22 (b) the factor "0.0676" was changed to "0.073."

(10) Under 37, line 2, the error in the size of sample was corrected to "0.2" instead of "0.02."

(11) A general volumetric method for the determination of maltose dextrose and lactose was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

XIV. FRUITS AND FRUIT PRODUCTS

(1) The tentative method for the determination of tartaric acid, p. 213, sec. 18, was dropped.

(2) The method for the determination of tartaric acid published previously[1] was adopted as tentative.

(3) The tentative method for the determination of citric acid, secs. 24 and 25, pp. 215 and 216, was dropped, and a method published previously[2] was adopted as tentative.

(4) The methods submitted by the referee for the determination of potash, manganese, calcium and magnesium were adopted as tentative.

(5) The tentative method for the determination of malic acid, secs. 19 to 23, inclusive, pp. 213–15, was dropped.

XV. CANNED VEGETABLES

(1) The tentative method "Physical Examination," sec. 1, p. 219, was revised as directed by the referee. (See *Methods of Analysis*, 1930.)

(2) The method, "Preparation of Sample—Official," sec. 2, p. 219, was amplified for the sake of clarity in regard to the kind and size of sieve used, as directed by the referee. (See *Methods of Analysis*, 1930.)

(3) The method, "Butyric Acid (qualitative test).—Tentative," sec. 25, p. 222, was dropped.

(4) The directions for the micro-analysis of tomato pulp, etc., secs. 27–30, p. 222, were rewritten to accord with the suggestions of the referee and approved by the committee. (See *Methods of Analysis*, 1930.)

XVI. CEREAL FOODS

(1) The method for sampling flour (official first action)[3] was adopted as official (final action).

(2) The present tentative method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent nitrogen in flour[4] was dropped, and the method proposed by the referee was adopted as tentative. (See *Methods of Analysis*, 1930.)

[1] *This Journal*, 13, 104 (1930).
[2] *Ibid.*, 99.
[3] *Ibid.*, 9, 39 (1926); 13, 73, Rec. (1) (1930).
[4] *Ibid.*, 40.

(3) The method for the determination of moisture in bread (official first action)[1] was adopted as official (final action).

(4) For the purpose of making the usual chemical determinations in bread one-half of the loaf as a sample was adopted as official (first action).

(5) The tentative method for the determination of fat in bread by acid hydrolysis[2] was made official (first action).

(6) The present tentative method of collecting and preparing a sample of alimentary paste for analysis[3] was dropped, and the following method was submitted as a tentative method:

Pick out sufficient strips to insure a representative sample and break them by hand into small pieces. Mix and grind 300–500 grams in a mill until all the material passes through a 20-mesh sieve. Keep the sample in a sealed container to prevent change in moisture content.

(7) The last paragraph of the method for the determination of total solids and moisture in alimentary pastes (tentative)[3] was deleted.

(8) In the official method for the determination of fat in flour by acid hydrolysis[4] the words "drying at the temperature of boiling water" were changed to "a drying oven at 98–105°."

XVII. MEAT AND MEAT PRODUCTS

(1) The following changes were made in the tentative method for the determination of added water in sausage and similar meat products:[5] Line 7, between the words "hours" and "or" the following phrase was inserted "or at a temperature of approximately 125°C. (not lower than 120° nor higher than 130°) for approximately 2–3 hours"; and in line 8, after the word "hours" the following phrase was added: "or 1 hour, respectively."

(2) In place of the first line of the tentative Sörensen method for the determination of amino nitrogen, sec. **34**, p. 248, the following directions were substituted: "To 20 cc. of the filtrate from **27**, neutralized to phenolphthalein with barium or sodium hydroxide or to 20 cc. of an equivalent extract of the."

(3) Secs. **37** and **38**, tentative, pp. 248–50, were deleted.

(4) The method for the determination of nitrites, sec. **14**, p. 240, was deleted, and a tentative method published previously[6] was substituted.

XVIII. GELATIN

(1) The tentative method for the determination of lead, sec. **8**, p. 256, was deleted.

(2) The alternative method for the determination of copper and zinc, sec. **10**, p. 256, was deleted.

[1] *This Journal*, **9**, 39 (1926); **12**, 40 (1929).
[2] *Ibid.*, **6**, 61 (1922).
[3] *Ibid.*, **9**, 43 (1926).
[4] *Ibid.*, 41.
[5] *Ibid.*, **13**, 42 (1930).
[6] *Ibid.*, **8**, 696 (1925).

(3) The diffusion method for the determination of sulfur dioxide, sec. 13, p. 257, was deleted.

(4) The chapter on gelatin will be incorporated in the chapter "Meat and Meat Products" in the 1930 revision of *Methods of Analysis*.

XIX. DAIRY PRODUCTS

(1) The official method for sampling butter (p. 275) was dropped (first action).

(2) The procedure for sampling tub and print butter and the type of container described by the associate referee were adopted as official (first action). (See *Methods of Analysis*, 1930.)

(3) The mechanical stirrer method published previously[1] was adopted as a tentative method.

(4) The microscopic method for the identity of malted milk[2] was adopted as tentative.

(5) In place of the tentative method for preparation of sample for malted milk, sec. 61, p. 275, which was adopted as tentative for dried milk,[3] the following method was adopted as tentative:

Sift the sample through a 20-mesh sieve onto a large sheet of paper, rubbing the material through the sieve and tapping vigorously if necessary. Grind the residue in a mortar, pass through the sieve, and mix into the sifted material, discarding all particles of wood and other material that cannot be ground. Sift the sample two more times, mixing thoroughly each time. To avoid absorption of moisture, operate as rapidly as possible. Preserve the sample in an air-tight container.

(6) The official Roese-Gottlieb method for the determination of fat, sec. 16, p. 262, was amended by omitting the first weighing of the flask, line 7, and requiring the use of a bead in the final extraction of the fat in petroleum ether to confirm the purity of the fat and by inserting a warning against wiping the flask just before weighing (first action).

(7) The filtering directions in the tentative method for the determination of casein,[4] modified last year,[5] were again changed to read as follows: "Add 0.5 gram of filter-cel, shake thoroughly, and filter clear through a suitable folded filter paper."

(8) Method II, official for the determination of casein, sec. 9, p. 260, was deleted (first action).

(9) Method II, official for the determination of albumin, sec. 11, p. 260, was deleted (first action).

XX. FATS AND OILS

(1) The combined Reichert-Meissl and Polenske method was made official and substituted for the present separate methods under "soluble"

[1] *This Journal*, 11, 276 (1928).
[2] *Ibid.*, 12, 238 (1929).
[3] *Ibid.*, 10, 35 (1927).
[4] *Ibid.*, 261, 10, 261 (1927).
[5] *Ibid.*, 13, 42 (1930).

and "insoluble volatile" acids (pp: 290–291), with the exception that the illustration of apparatus on p. 292 was retained (final action).

(2) The Kirschner method[1] for the determination of volatile acids, the silver salts of which are soluble, using standard solutions of sodium, potassium, or barium hydroxide for the titration, was adopted as official (final action).

(3) The official Leffman and Beam method, secs. 28 and 29, p. 291, was deleted (first action).

(4) Reagent (c), in the revised Reichert-Meissl and Polenske Values was changed to read, "(b) Standard 0.1 *N* sodium or potassium hydroxide solution," and in the determination "5 cc." was changed to "6 cc."

(5) Sec. 30, p. 291 was changed slightly as recommended by the referee. (See *Methods of Analysis*, 1930.)

XXI. BAKING POWDERS AND BAKING CHEMICALS

(1) The official qualitative test for the determination of aluminum in the presence of phosphates, sec. 22, p. 308, was deleted (first action), and the test submitted by the referee was substituted as a tentative method. (See *Methods of Analysis*, 1930.)

(2) The Reynolds, Ross, Jacob method for the determination of fluorides[3] was substituted for the present tentative method of determining fluorides in baking powder, secs. 35–38, pp. 312–14, including the figure.

XXII. SPICES AND OTHER CONDIMENTS

The method submitted by Mitchell and Alfend, slightly modified, for the determination of the iodine number of paprika oil,[4] was adopted as tentative.

XXIII. VINEGARS

(1) The tentative directions for polarization (sec. 23, p. 329) were changed to read as follows:

Whenever possible, polarize in a 200 mm. tube without decolorizing. Report results on the basis of a 200 mm. tube in degrees Ventzke. When necessary decolorization may be accomplished in the following manner:

(a) To 50 cc. of the sample add a measured quantity of saturated neutral lead acetate solution, avoiding an excess of lead, and filter; remove the lead with powdered anhydrous potassium oxalate and filter. Polarize and correct for the dilution with lead acetate solution.

(b) To 50 cc. of the sample add decolorizing carbon, avoiding an excessive amount or length of treatment. Filter through a double paper and polarize.

(2) The first line of the method for the determination of sulfates, sec. 24, p. 329, was changed to read as follows: "To 100 cc. of the absolutely

[1] *This Journal*, 13, 44 (1930).
[2] *Ibid.*, 43.
[3] *Ibid.*, 11, 231 (1928).
Ibid., 524.

clear sample," and the method so changed was adopted as official (first action).

(3) The method for "Alcohol Precipitate.—Tentative," sec. 26, p. 330, was dropped.

(4) The method for "Pentosans.—Official," sec. 27, p. 330, was dropped (first action).

(5) The method for "Lead Precipitate.—Tentative," sec. 22, p. 329, was deleted.

(6) Under "Alkalinity of the Insoluble Ash," sec. 7, change "0.1 *N* HCl" to "*N* HCl."

XXIV. COFFEES

No additions, deletions, or other changes.

XXV. TEAS

On p. 340, sec. 17, delete the last paragraph and substitute the material submitted by the referee (first action). (See *Methods of Analysis*, 1930.)

XXVI. CACAO PRODUCTS

(1) The modified method submitted by the referee for the determination of crude fiber in bitter and sweet chocolates was adopted as official (first action). (See *Methods of Analysis*, 1930.)

(2) The following method for the determination of moisture was adopted as a tentative method:

Dry 2 grams of the sample, prepared as directed under (1), p. 343, in a platinum dish in an air oven at 100°C. (An aluminum dish may be used when ash is not determined on the same sample.) Report the loss in weight as moisture.

(3) The third paragraph under sec. 19, "Determination," p. 346, was changed by inserting the phrase, "using 5 grams of fat," at the end of the first sentence (line 3).

(4) The phrase "by more than 2 degrees" was deleted from line 6 of the same paragraph.

(5) The following words were inserted between the word "butter" and the word "adulteration" in the 6th line of the same paragraph: "by more than 3 degrees in the case of fat from chocolate liquors or sweet chocolate and by more than 6 degrees in the case of fat from milk chocolate."

(6) The heading "Reichert-Meissl Number.—Official" and the heading "Polenske Number.—Official," secs. 26 and 27, p. 347, were combined to read "Reichert-Meissl and Polenske Values.—Official," and appropriate reference changes were made in the details of the method in the chapter on Fats and Oils.

(7) The directions for the separation and preparation of fat, sec. 16, p. 345, were modified by the referee.

XXVII. FLAVORING EXTRACTS

(1) The title of this chapter was changed to "Flavors and Non-alcoholic Beverages."

(2) The official Kleber method for the determination of citral (p. 355) was removed from its place under the heading "Lemon and Orange Oils—Citral" and placed under the heading "Lemon and Orange Oils—Total Aldehydes" (final action).

(3) On p. 350, sec. 6, in the title the word "Winton" was inserted after the word "Number."

(4) The Wichmann method for the determination of the lead number[1] was changed by deleting reagent (b) and also the last paragraph under "Determination," as suggested by the referee. (See *Methods of Analysis*, 1930.)

(5) The qualitative test for vanilla resins, p. 350, sec. 11, was changed as suggested by the referee.[2] (See *Methods of Analysis*, 1930.)

XXVIII. WINES

No additions, deletions or other changes.

XXIX. DISTILLED LIQUORS

(1) In lines 1 and 2 of the method for the determination of esters, sec. 8, p. 370, the quantity "200 cc." was changed to "100–200 cc." and in line 1 the quantity "25 cc." was changed to "12.5–25 cc." (first action).

(2) In the official method for the determination of fusel oil, sec. 14, p. 371, in lines 1 and 2, "100 cc.," was changed to "50 cc." and after the word "sample," line 1, and the word "liquid," line 2, the phrase "add 50 cc. of water then" was inserted (first action).

(3) Par. 5, sec. 14, was rewritten according to the recommendation of the referee. (See *Methods of Analysis*, 1930.)

(4) Under "Caramel," sec. 23, p. 374, were inserted a new test, entitled "Marsh's Test for Artificial Colors" and a method for the detection of methanol in alcoholic beverages.

XXX. BEERS

No additions, deletions, or other changes.

XXXI. DRUGS

(1) The method submitted by the associate referee for the determination of calomel in calomel ointment was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(2) The microchemical tests for the identification of atropine and pilocarpine,[3] now tentative, were adopted as official (first action).

[1] *This Journal*, 9, 47 (1926).
[2] *Ibid.*, 48.
[3] *Ibid.*, 11, 354 (1928); 12, 284 (1929).

(3) The microchemical method for the identification of ephedrine submitted by the associate referee was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(4) The gravimetric method recommended by the associate referee for the assay of santonin in mixtures and tablets was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(5) The method recommended by the associate referee for the bioassay of fluidextract of ergot was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(6) The cat-eye method for the assay of mydriatic and myopic drugs, adopted as tentative in 1927,[1] was adopted as official (first action).

(7) The present tentative method for the assay of ephedra, amended by deleting titration procedure No. 1,[2] was adopted as an official method (first action).

(8) The quantitative method for the determination of ephedrine in tablets[3] was amended by substituting for the section between "see discussion" and "convenient for a control" the following directions; and as so amended was made tentative:

Remove from the steam bath and add bromthymol blue indicator and a measured excess of 0.02 *N* sulfuric acid. Add about 40 cc. of carbon dioxide-free water, cover with a watch-glass, return to the bath to dissolve the alkaloid adhering to the sides of the flask, and evaporate all the ether. Titrate the excess acid with 0.02 *N* sodium hydroxide, using standard indicator, *p*H 6.0, for comparison.

1 cc. of 0.02 *N* sulfuric acid = 0.0033 gram of ephedrine.

(9) A qualitative color test for the identification of ephedrine by means of copper sulfate-sodium hydroxide was adopted as a tentative method.[4]

(10) The melting point determination for the identification of ephedrine, p. 163, sec. **51**; *This Journal*, **13**, 330 (1930), was adopted as tentative.

(11) The method submitted by the associate referee for the determination of ephedrine in inhalants was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(12) The method for the determination of menthol described by the associate referee and published[5] last year was adopted as a tentative method.

(13) The Paget method for the determination of oil of chenopodium[6] was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(14) The method submitted by the associate referee for the determination of emetine hydrochloride in tablets was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

[1] *This Journal*, **10**, 383 (1928); **11**, 53 (1928).
[2] *Ibid.*, **12**, 291 (1929).
[3] *Ibid.*, **13**, 329 (1930).
[4] *Ibid.*, 330.
[5] *Ibid.*, **12**, 300 (1929).
[6] *Ibid.*, **13**, 336 (1930).

(15) The method of Roberts and Murray[1] for the determination of chloroform in mixtures, modified by the associate referee, was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(16) The method described by the associate referee for the determination of iodoform was adopted as a tentative method. (See *Methods of Analysis*, 1930.)

(17) The methods for sampling drugs,[2] as modified by the referee, were adopted as tentative.

(18) In the method for the determination of barbital and phenobarbital[3] the following phrase was inserted: "Melting Point (use U. S. P. Method)."

(19) The tentative method for the determination of ipecac[4] was modified by the addition of 60 cc. of water, as suggested by the referee. (See *Methods of Analysis*, 1930.)

(20) Reagent **(a)** of the tentative method for the determination of acetanilid, sec. **4**, p. 381, was modified to read "15 grams of potassium bromate" instead of "3 grams" and "100 grams" of potassium bromide instead of "50 grams."

(21) Under sec. **24**, p. 388, the following directions were substituted for reagent **(c)**: "Dissolve 3 grams of potassium bromate and 50 grams of potassium bromide. Dilute to 1 liter and standardize 30 cc. of the solution, using a 500 cc. Erlenmeyer flask, following the procedure under **25**, beginning 'and 500 cc. of hydrochloric acid'."

(22) The nitrate and nitrite methods for the determination of nitroglycerin in tablets, secs. **45–50**, were deleted.

(23) The yeast method for the determination of ionic silver was deleted.

(24) The single titration method (a), now tentative for the determination of acetylsalicylic acid was dropped.[5]

(25) The following tentative methods were made official (first action): The melting point determination of acetylsalicylic acid, sec. **19**, p. 387; the method for the determination of camphor;[6] Methods I and II for the determination of nitroglycerin;[7] the quantitative method for the determination of pyramidon;[8] Method II for the determination of cocaine[9] (to be published as Method I); method for the determination of arsenic in iron methylarsenate;[9] method for the determination of calomel in tablets;[10] the microchemical methods for the following alkaloids: atropine, brucine, caffeine, cinchonidine, cinchonine, cocaine, codeine, morphine, pilocarpine, quinidine, quinine, strychnine.[11]

[1] *Am. J. Pharm.*, **101**, 654 (1929).
[2] *This Journal*, **10**, 99 (1927).
[3] *Ibid.*, **8**, 48, 510 (1925); **9**, 51 (1926).
[4] *Ibid.*, **11**, 50 (1928).
[5] *Ibid.*, **9**, 49 (1926).
[6] *Ibid.*, 52.
[7] *Ibid.*, **10**, 47 (1927).
[8] *Ibid.*, **11**, 51 (1928).
[9] *Ibid.*, 49
[10] *Ibid.*, 51; **12**, 52 (1929).
[11] *Ibid.*, **11**, 52, 354 (1928); **12**, 53 (1929); **13**, 46 (1930).

EGGS AND EGG PRODUCTS

(1) The method submitted by the referee for the determination of total phosphoric acid (P_2O_5) was adopted as tentative. (See *Methods of Analysis*, 1930.)

(2) The method submitted by the referee for the determination of chlorine was adopted as tentative. (See *Methods of Analysis*, 1930.)

(3) Par. **(b)**, "Apparatus," of the tentative 98°C. vacuum oven method for the determination of total solids (official first action)[1] was changed to read as follows: **(b)** *Air-tight desiccator.*—Should contain a fresh efficient desiccator" and adopted as official (final action).

(4) The tentative routine 112°–117°C. air-oven method (II) for the determination of total solids[2] was dropped.

(5) The tentative method for the determination of ash in eggs[3] was dropped.

NAVAL STORES

(1) The methods that pertain to naval stores, formerly published under the chapter "Drugs," were assembled to be published under a new separate chapter, entitled "Naval Stores."

(2) The method for specific gravity, p. 405, sec. **78**, in its relation to naval stores, was deleted and the method submitted by the referee was substituted. (See *Methods of Analysis*, 1930.)

RADIOACTIVITY OF FOODS AND DRUGS

(1) The methods that pertain to the radioactivity of foods and drugs were assembled to be published under a new separate chapter.

(2) Under "Standard radium solution," in the method for the determination of radioactivity of water[4] the quantity "100 cc." was changed to "200 cc." and the quantity "80 cc." to "160 cc." Other editorial changes were made for the sake of clarity. (See *Methods of Analysis*, 1930.)

(3) The methods for the preparation of radioactive samples,[5] were adopted as official (first action).

(4) The tentative methods for the calibration of electroscopes and for the analysis of radioactive samples[4] were adopted as official (first action) after revision as suggested by the referee.

CAUSTIC POISONS

(1) Method I (Chapin) for the determination of phenol (carbolic acid) in such products as cresol, saponified cresol solutions, coal tar dips, disinfectants, etc., was adopted as an official method (final action). The method has been published.[6]

[1] *This Journal*, 9, 56 (1926).
[2] *Ibid.*, 57.
[3] *Ibid.*, 12, 55 (1929).
[4] *Ibid.*, 8, 531 (1925).
[5] *Ibid.*, 10, 362 (1927).
[6] U. S. Dept. Agr. Bull. 1308 (1924); *This Journal*, 13, 49 (1930).

(2) Method II (Hamilton and Smith modification of Chapin method) for the determination of phenol (carbolic acid) in the presence of methyl salicylate in such products as fly sprays, disinfectants, etc., was adopted as an official method (final action).

PAINTS

(1) The methods for the routine analysis of white linseed oil paints (D 215–29 of the Am. Soc. for Test. Mats.) were adopted as tentative methods to be printed as a separate chapter entitled "Paints" in the 1930 revision of *Methods of Analysis*.

REPORT OF REPRESENTATIVES ON THE BOARD OF GOVERNORS OF THE CROP PROTECTION INSTITUTE OF THE NATIONAL RESEARCH COUNCIL

Most of the work of the Board of Governors of the Crop Protection Institute is conducted through correspondence. Before contracts are made the Chairman of the Board submits details regarding every project to be undertaken to the members for their suggestions and approval. The Chairman also makes written reports to each member from time to time as to the progress being made on the various projects. This gives each member close contact with the work.

Dr. MacIntire attended the annual meeting of the Board of Governors, held at Des Moines, Iowa, in December, 1929.

The following projects are being conducted this year:

1. The use of Volck for animal parasites at Manhattan, Kansas, for the California Spray-Chemical Corporation.
2. A study of oils for the codling moth at Pullman, Washington and Berkeley, California, for the California Spray-Chemical:Corporation.
3. A study of oil sprays for the oriental fruit moth at Newark, Delaware, for The California Spray-Chemical Corporation.
4. A study of oil residues in plant tissues at two points, one on the Atlantic Coast and one on the Pacific Coast, for The California Spray-Chemical Corporation.
5. The horticultural uses of adhesive tape at Madison, Wisconsin, for Johnson and Johnson.
6. The incorporation of fungicides in oxidized oils at Newark, Delaware.
7. Flotation sulfur project at Urbana, Illinois, for Koppers Company.
8. Pyrethrum extracts at Columbus, Ohio, for J. C. Makepeace.
9. The insecticide and fungicide properties of sulfur-bearing oils at Urbana, Illinois, for the Midwest Chemical Company.
10. Household insecticides at Ames, Iowa, for the Deep Rock Oil Corporation.
11. Horticultural oil sprays at Urbana, Illinois, for Standard Oil Company of Indiana.
12. The incorporation of fungicides in oil sprays at New Brunswick, New Jersey, for Standard Oil Co. of N. J.
13. The development of impregnated oils at New Haven, Connecticut, for Standard Oil Co. of N. J.

14. Methods and localities suited for the growing of pyrethrum at Lexington, Kentucky, for the Standard Oil Co. of N. J.
15. Copper sprays at Wooster, Ohio, for the Tennessee Copper and Chemical Co.
16. Chemical compounds derived from coconut oils, at Boyce Thompson Institute, for the Research Corporation.

From this list it will be seen that the investigations cover a wide range of subjects and that they are located in all parts of the United States. These projects are of great interest to the chemists as their ultimate solution necessitates much chemical research.

This list would also indicate that the Crop Protection Institute is filling a much needed and valuable position in serving as a neutral unbiased agency for conducting research on commercial products which are of interest to the public and also for making the trained research workers and facilities at our State institutions of greater service.

Your representatives would be glad to receive suggestions or instructions from this Association as to matters which you would like to have presented to the Board of Governors of the Crop Protection Institute.

H. J. PATTERSON
W. H. MACINTIRE

Approved

REPORT OF SECRETARY-TREASURER

Only one resignation of a referee was received during the year, and that was owing to a change in position. E. M. Bailey, W. W. Skinner, and L. E. Warren were appointed delegates to the U. S. P. Convention held May 13, 1930, and H. R. Watkins and L. E. Warren represented the association at the National Conference on Pharmaceutical Research. W. F. Hand was appointed to fill the vacancy on the Committee on Necrology occasioned by the death of W. W. Randall.

At the meeting of the Executive Committee certain actions were taken merely as a matter of routine within the province of its activities as the steering committee of the association. The committee suggested that the Board of Editors continue periodically to solicit bids for their work in order to be sure that the association is getting the best possible services at the lowest cost. As Secretary, I shall bring in next year a report of the advisability of incorporating this association; it has been discussed in the past, but no definite action has been taken. The business activities of the association would seem to make it advisable to incorporate. The treasurer is rather apprehensive if he has to stand responsible for all the association bills, to the extent of nearly $10,000. The chairman of the Board of Editors has reported, and I should like to make a record here of the prospect of enlarging the *Journal* to correspond with the leading food and analytical journals of Europe. The executive committee approves this idea of

bringing the *Journal* of the association in harmony with the type of journal that is being supported in European countries.

One of the other matters that were considered by the Executive Committee is the need of standardized biological methods. At the time at our disposal we could not reach a decision on this matter, but it is apparent that this association needs to branch out in that field if it is to serve fully the needs of its members. The matter of microchemical methods has been before the association on numerous occasions. We heard Dr. Browne's interesting talk yesterday about what is going on in Europe, and if we are going to be at the front of the procession it will be necessary for the executive committee this coming year to devote considerable attention to microchemical analysis.

I will pass on rapidly to the treasurer's report, which ought to be a matter of record.

RECEIPTS—METHODS OF ANALYSIS AND JOURNAL

Methods of Analysis

Number	Price each	
12	$5.50	$66.00
230	5.00	1,150.00
72	4.40	316.80
158	4.00	632.00
Total		$2,164.80

Journal Subscriptions

Number	Price each	
1	$7.50	$7.50
2	6.00	12.00
39	5.50	214.50
361	5.00	1,805.00
5	4.50	22.50
118	4.40	519.20
243	4.00	972.00
1	3.50	3.50
2	2.00	4.00
9	1.50	13.50
1	1.40	1.40
		$3,575.10
Minus charge for exchange		2.18
Total		$3,572.92

Advertisements

Number	Price each	
4	$15.00	$60.00
11	25.00	275.00
Total		$335.00

Miscellaneous

Interest from bankruptcy account	.33

Reprints

G. A. Shuey, Knoxville, Tenn.	$ 2.09	
L. E. Warren, Washington, D.C.	7.25	
H. A. Schuette, Madison, Wis.	4.96	
M. J. Blish, Lincoln, Neb.	4.13	
H. R. Kraybill, Lafayette, Ind.	3.00	
Amer. Pharmaceutical Mfgrs., Des Moines, Iowa	3.19	
W. L. Hill and K. D. Jacob	5.00	
R. E. Remington, Charleston, S.C.	22.50	
J. S. McHargue, Lexington, Ky.	4.18	
J. B. Smith, Wakefield, R.I.	4.93	
F. W. Zerban, N.Y.C.	11.30	
J. F. Snell, Montreal, Quebec	19.00	
A. L. Prince, New Brunswick, N.J.	5.96	
Total		$97.49

Total for *Methods*, *Journal*, Ads., Reprints and Miscellaneous	$6,170.54
Plus foreign collections	15.30
Bank balance of October 15, 1929	1,746.65
Total	$7,932.40

DISBURSEMENTS

1929		Amount	Check Number
Oct. 14	Bastian Bros. Co., name bars for meeting	$ 30.32	314
31	J. J. Betton, premium on bond, M. A. Bates	2.50	315
Nov. 25	Industrial Printing Co., bill of 10–14–19, letter heads.	3.50	316
30	Marie-Alice Bates, office expenses	50.00	317
30	Bastian Bros. Co., name bars	16.09	318
Dec. 17	Ace Letter Service, copies of Constitution A.O.A.C.	9.80	319
17	H. F. Warneson, binding 24 vols. of *Journal*	48 00	320
26	Industrial Printing Co., bill of 11–3–29, reprints	7.25	321
26	Industrial Printing Co., bill of 12–17–29, reprints	20.25	322
26	Postmaster, Wash., D.C., quarter ending, 12/31/29	2.00	323
1930			
Jan. 10	Industrial Printing Co., bill 11/30/29, *Journal*, Vol. XII, No. 4	955.34	324
10	Refund to Assn. account, dues S.C. Food Res. Comm.	5.00	325
29	Marie-Alice Bates, office expenses	50.00	326
Feb. 10	Dept. of Agriculture, Canada, refund on subscription.	3.00	327
18	Postmaster, Washington, D.C., mailing *Journals*	25.00	328
Mar. 21	Postmaster, Wash., D.C , quarter ending 3/31/30	2.00	329
21	Industrial Printing Co., bill 2/6/30, letter heads	11.75	330
25	Marie-Alice Bates, office expenses	50.00	331
Apr. 22	Industrial Printing Co., bill of 3/29/30, *Journal*, Vol. XIII, No. 1	1,208.84	332
May 10	Delecta Ltd., Watford, Eng., refund on subscription.	5.50	333

	21	Cash, dues for Massachusetts Agriculture College....	5.00	334
June	2	Marie-Alice Bates, office expenses.....	50.00	335
	23	Postmaster, Wash., D.C., quarter ending 6/30/30....	2.00	336
	30	Industrial Printing Co., bill of 5/31/30, *Journal*, Vol. XIII, No. 2.....	1,231.98	337
	30	Industrial Printing Co., bill of 6/19/30, letter heads..	29.50	338
July	9	Refund to Assn. account, dues Kansas Agricultural College.....	5.00	339
	9	Industrial Printing Co., bill of 6/23/30, reprints.....	80.60	340
	10	Refund to Assn. acct., dues Kansas Agr. College....	5.00	341
Aug.	12	Marian E. Lapp, office expenses.....	50.00	342
	22	Industrial Printing Co., fire insurance.....	2.10	343
Sept.	4	Industrial Printing Co., bill of 8/27/30, *Journal*, Vol. XIII, No. 3.....	1,012.63	345
	4	Industrial Printing Co., bill of 3/29/30, reprints.....	89.20	346
	11	Refund to Assn. acct., dues of Maryland Dept. of Health.....	5.00	347
	25	Postmaster, Wash., D.C, quarter ending 9/31/30....	2.00	348
		Plus bank balance, October 1, 1930.....	2,856.25	
		Total.....	$7,932.40	

STATEMENT OF OPERATING ACCOUNT

October 15, 1929, to October 1, 1930

RECEIPTS—ASSOCIATION

1929			
Oct.	15	Bank balance.....	$260.77
		1929 dues from institutional members, 69 at $5.00....	345.00
			$605.77

DISBURSEMENTS

1929				Check No.
Oct.	21	Marian E. Lapp, expenses, 1929 meeting.....	$40.00	78
1930				
Apr.	5	Gude Bros., flowers for B. B. Ross.....	16.12	79
June	30	Joseph Cohen, affidavits.....	5.00	80
July	31	A. S. Mitchell, flowers for W.W. Randall.....	10.00	81
Aug.	1	Gude Bros., flowers for H. W. Wiley.....	15.00	82
	22	Stamps for mailing programs.....	15.00	83
Sept.	4	Industrial Printing Co. bill 8–22–30, programs.....	37.75	84
			$138.87	
Oct.	1	Bank balance.....	466.90	
		Total.....	$605.77	

SUMMARIZED STATEMENT

ASSETS

Operating account balance.....	$466.90
Publications account balance.....	2,856.25
Savings account (Montgomery Bldg. and Loan Assn.).....	1,520.93
Total assets.....	$4,844.08

LIABILITIES

Bills payable—Reprints, Vol. XIII, No. 3	$52.75
Balance	$4,791.33

This showing, I think, is an unusually good one considering the difficulties which we have had to meet within the past five years. The *Book of Methods* is financing the association. Of course we shall have to advance considerable money when the first delivery is made of the new revision. We have—and I think we can say this with some pride—$4,791.33 in cash, assets with which to anticipate the advances we shall have to make in meeting the first expenses of the revised *Book of Methods.*

W. W. SKINNER

Approved

REPORT OF COMMITTEE TO COOPERATE WITH OTHER COMMITTEES ON FOOD DEFINITIONS

This committee respectfully submits the following report covering the proceedings of the Food Standards Committee during the past year.

Since the 1929 convention of this association but one meeting of the Committee has been held, this occurring during the week of April 28. The two main topics to receive consideration and which consumed the greater part of the time of this session were definitions and standards for wheat flour and for various types of beverages and beverage concentrates.

At the previous meeting considerable time had been devoted in a preliminary way to the problem presented by the long existent confusion as entailed by the loose use on the part both of the milling industry and consumers of the terms "whole wheat flour," "entire wheat flour" and "graham flour."

At that time tentative definitions for these products and also for white flour were formulated and put out to the trade and to the public for comment. During the interim between the two meetings such comments were quite freely submitted, it being perhaps interesting to note that these included over two thousand letters addressed to the chairman of the Committee from Eastern consumers. These appeals to the committee for action were inspired by a radio campaign conducted by a certain food publicist.

At the April meeting the committee gave a public hearing on this subject. This meeting, held in the assembly hall of the National Museum, brought out a large attendance on the part of the milling industry and the public, and great interest was manifested. The definitions and standards for these flours as finally adopted by the committee for the approval of the Secretary of Agriculture are as follows:

Whole wheat flour, entire wheat flour, graham flour, is the clean, sound product made by grinding wheat, and contains in their natural proportions, all of the constituents of the cleaned grain.

Flour, wheat flour, white flour, is the clean, sound, fine-ground product obtained in the commercial milling of wheat, and consists essentially of the starch and gluten of the endosperm. It contains not more than 15 per cent of moisture, not less than 1 per cent of nitrogen, not more than 1 per cent of ash, and not more than 0.5 per cent of fiber.

Bolted whole wheat flour, bolted entire wheat flour, bolted graham flour, is the clean sound product made from wheat by grinding and bolting, and contains all of the grain except a portion of the bran.

At previous meetings some progress had been made in developing definitions for beverages, and at this time the matter received intensive consideration, definitions for fruit juices being adopted as follows:

Fruit juice is the clean, unfermented liquid obtained from the first pressing of sound, ripe, fresh fruit, or of its pulp, and conforms in name to the fruit from which it is obtained.

Grape juice is the clean, unfermented juice of sound, ripe grapes. It is obtained by a single pressing of the fruit, with or without the aid of heat, and with or without the removal of insoluble matter.

Orange juice is the clean, unfermented juice obtained from sound, ripe sweet oranges. It may contain a portion of the pulp and/or of the volatile oil.

Tentative definitions for carbonated beverages and concentrates and flavors therefor were developed at this meeting and submitted to the industry for criticism. These included root beer, birch beer, cream soda water, orange soda water, lemon soda water, and lime soda water, with definitions for the corresponding flavors or concentrates.

In such connection one of the proposals of some elements in the industry is that the sweetening agent be not limited to sugar (sucrose), but that the use of invert sugar and of refined corn sugar (dextrose) be also recognized. This is one of the matters which will receive future consideration by the committee. It is believed that the establishment of these definitions will mark an important step forward in the regulation of the beverage industry.

Another phase of this matter, and one which has already been the subject of some study and debate by the committee, involves definitions and standards for the type of beverage now very largely distributed and which involves dilutions of fruit juices with water, either plain or carbonated. These beverages, now being commonly offered by the bottler under such names as "ade," "squeeze" and "drink," in combination with the name of the fruit, have already become quite a problem with the regulatory official, the principal question involved by them being the minimum quantity of fruit juice which should be present to entitle to such names.

At present there is no recognized standard or practice as to the composition of these fruit-flavored drinks, the result being that the quantity of fruit juice may vary from as much as 15 or 20 per cent down to practically

nothing. An abuse in this connection which has sprung up during the past year is the incorporation in some of these citrous drinks of a quantity of inert pulp for the purpose of imparting a rich fruity effect, although the flavor may actually be due mainly or wholly to essential oil. In the case of "orangeade" it appears that a tentative minimum working limit recognized in some states, is, or has been, as little as 5 per cent of juice, in others 10 per cent is being required, and in one or two 15 per cent has been fixed. Thus far a majority of the states, in view of the lack of a standard, have not ventured to attempt any regulation of this feature. It is hoped that such standards, acceptable to the industry and to regulatory officials, can be developed by the committee in the near future.

It will be recalled that in past years the committee devoted considerable effort to an attempt to formulate what it conceived as being a proper definition and standard for ice cream, but that it found itself so handicapped both by the attitude of the industry and the existing differences in state statutory standards that it eventually found itself seemingly obliged to abandon the problem—at least for the time being. However at this time the situation seems to be a bit more encouraging. Not only do state standards show an upward tendency but the industry is now seemingly manifesting a more liberal and broader-minded attitude toward this matter than formerly. As suggestive of the latter, it is worthy of note here that at the last meeting the committee gave brief consideration to an appeal addressed to it by the secretary of the manufacturers' organization asking that the subject of definitions and standards for this product be revived.

It appears that the definitions and standards recently adopted by the committee for mayonnaise are meeting with some objection on the part of certain elements in this industry. Requests for modification received from two or three of these manufacturers came up for brief consideration at this meeting.

C. D. HOWARD
E. M. BAILEY
G. G. FRARY

Approved

No report was given by the Chairman of the Committee on Sampling.

No report was given by the Chairman of the Committee on Bibliography.

REPORT OF AUDITING COMMITTEE

The Auditing Committee has examined the accounts of *The Journal* and *Methods of Analysis*, covering the period from October 15, 1929, to October 1, 1930, and found the same to be correct.

The Committee has also examined the accounts of W. W. Skinner, Secretary-Treasurer, covering the period from October 15, 1929, to October 1, 1930, and found the same to be correct.

A. S. MITCHELL
G. G. FRARY
L. H. BAILEY

Approved

REPORT OF COMMITTEE ON NECROLOGY

The past year has been the darkest in the history of our association for the number and prominence of the members who have been removed by death. The roll is a long and illustrious one. It includes the names of:

(*1*) *H. W. Wiley*, charter member, past president, past secretary and honorary president, with the unique record of having attended each one of our annual meetings for a continuous period of 44 years. His loss is irreparable. The services which he rendered to our association and to all mankind were fully set forth in the tributes paid yesterday to his memory, all of which will appear in a forthcoming issue of our *Journal*. (Published in this number.)

(*2*) *B. B. Ross*, past president of our association, a constant attendant for nearly 40 years at its annual meetings and a most efficient, conscientious worker in all the fields of its activity. His services to this association were faithful and of the very highest value. An account of his life work was published in an obituary by W. W. Skinner in the *Journal* for August, 1930.

(*3*) *W. W. Randall*, past president of our association, to which he rendered most valuable services in many capacities by his ability and scholarly attainments. His professional career and high merits have also been commemorated in a biographical sketch by F. C. Blanck which appeared in the *Journal* for November, 1930.

(*4*) *J. B. Weems*, chief chemist of the Virginia Department of Agriculture, and a regular attendant for the past 15 years at the meetings of our association, to which he rendered most valued service. His obituary by W. C. Jones was published in the *Journal* for May, 1930.

(*5*) *E. A. Read*, a highly skilled microscopist and a food expert of outstanding ability. During her 24 years of faithful service in the U. S. Bureau of Chemistry and the Food and Insecticide Administration, Dr. Read was a regular attendant at the meetings of our association, in whose work she participated as collaborator and referee. She was a graduate of Cornell University from which she held both the B.S. and Ph.D. degrees. She held also the degree of M.D. conferred by George Washington University. Dr. Read is best known to our membership for the color test which she originated for detecting the adulteration of tea. A woman of most charming personality, who was imbued with the deepest devotion to her work,

she will long be held in loving remembrance by her many friends and scientific associates.

(6) *L. E. Dawson,* carbohydrate chemist and technologist of the U. S. Bureau of Chemistry and Soils. He was a frequent attendant at the meings of our association, which he served in the collaborative work upon methods of sugar analysis. His early death marks the termination of one of the most promising careers in the field of agricultural chemical technology.

(7) *J. W. Barnes,* chemist of the U. S. Bureau of Chemistry and Soils who greatly assisted our association by his collaborative work upon methods for the analysis of insecticides. He was a most painstaking analyst and his early passing removes from our association another younger member of great ability and promise.

We cannot hope to replace these departed comrades and coworkers, but let us who follow in their footsteps show the reverence in which we hold their names by striving to emulate their worth and example. I move you Mr. President that we all rise as a token of respect for their memory.

C. A. BROWNE

Approved

REPORT OF NOMINATING COMMITTEE

The Nominating Committee desires to place in nomination the following names:

President: H. D. Haskins, Agricultural Experiment Station, Amherst Mass.

Vice-President: A. E. Paul, Food and Drug Adm., Chicago, Ill.

Secretary-Treasurer: W. W. Skinner, Bureau of Chemistry and Soils, Washington, D. C.

Additional Members of the Executive Committee:

J. W. Kellogg, Harrisburg, Pa.
F. C. Blanck, Washington, D. C.
R. Harcourt, Guelph, Canada
E. M. Bailey, New Haven, Conn.

W. H. MACINTIRE
C. S. CATHCART
R. N. BRACKETT

It was moved, seconded and carried that the secretary be directed to cast a unanimous ballot for the officers nominated.

REPORT OF COMMITTEE ON RESOLUTIONS

The sorrow brought to the members of this association at the meeting last year by the announcement that our honorary president could not

meet with us was deepened in the days that followed by the knowledge that in his physical presence he had passed from us. But the spirit he brought to us at our meetings will not fade quickly, for we believe we shall go on in the future years inspired by that enthusiasm in truth seeking which ever characterized him. It is entirely fitting that this organization has paused in the midst of its annual business session to give expression of our appreciation of his life associations and work. The addresses of these worthy associates of Dr. Wiley constitute a valuable historical record and worthy tribute to our honorary president which will be treasured by his friends and the members of this association. These serve as the expression of the worthful praise of your committee to Dr. Wiley in a far more complete and excellent way than could be attempted in this report. Therefore be it

(*1*) *Resolved:* That we express our hearty appreciation of the program honoring Dr. Wiley and our thanks to each individual speaker, to the committee who planned it and to all others who have contributed to its success.

(*2*) *Resolved:* That we indorse the words of tribute paid by the Committee on Necrology to other worthy members who in like manner have left us. The names are B. B. Ross, W. W. Randall, Effie A. Read, J. B. Weems, Louis E. Dawson and J. W. Barnes. We also suggest that a copy of these tributes be sent to the family of each deceased member.

(*3*) *Resolved:* That we express our appreciation and thanks to Dr. A. F. Woods, Director of Scientific Work, U. S. Department of Agriculture, for his direct and hearty words of greeting, which were so full of encouragement for the officers and members of the association.

(*4*) *Resolved:* That the thanks of this association be extended to our retiring president, Dr. E. M. Bailey, for the unselfish service gladly rendered to the association during the year just past and for his informing presidential address on the historical beginnings of our organization work.

(*5*) *Resolved:* That in the publication of our *Journal* we are indebted to Mr R. B. Deemer for his careful work as editor and render him our vote of thanks.

(*6*) *Resolved:* That in Dr. W. W. Skinner, secretary-treasurer of the association and editor in charge of revision of *Methods of Analysis*, we have a faithful working officer, whose services are indispensable and whose fidelity is sincerely appreciated. Again we say to him as heartily as we can express it, "Thank you many times."

(*7*) *Resolved:* That this association greatly appreciates the efficient work and gracious spirit of Miss Lapp, who with her capable assistants has contributed greatly to the success of our annual meeting and to the perfection of the published *Journal* of the association. To her and her helpers we acknowledge our debt of gratitude and express our heartfelt thanks.

(8) *Resolved:* That the management of the Raleigh Hotel be granted a vote of thanks for the accommodations and conveniences placed at the disposal of our association and for the many courtesies extended individually to the members in attendance at this annual meeting.

L. D. HAIGH
C. A. BROWNE

Approved

The association also approved the following resolutions relating to the George Washington Bicentennial:

WHEREAS, The Congress of the United States has created a Commission to arrange a fitting nation-wide observance of the two hundredth anniversary of the birth of George Washington in 1932, and

WHEREAS, The Commission so created, composed of the President of the United States, the Vice-President of the United States, the Speaker of the House of Representatives, four members of the United States Senate, four members of the House of Represetatives and eight citizens appointed by the President of the United States, is charged with the duty of planning and directing the celebration, and

WHEREAS, The high purpose of the event is to commemorate the life, character and achievements of the most illustrious citizen of our Republic and to give every man, woman and child living under the Stars and Stripes an opportunity to take part in the celebration which will be outstanding in the world's history, and

WHEREAS, The George Washington Bicentennial Commission, desiring the full cooperation of the people in the United States, has extended a most cordial and urgent invitation to our organization to participate in the celebration, therefore be it

RESOLVED, That the Association of Official Agricultural Chemists does hereby endorse the program of observance of the two hundredth anniversary of the birth of George Washington, to take place in 1932; accept with appreciation the invitation of the George Washington Bicentennial Commission, and pledge this organization to extend earnest cooperation to the United States Commission in all possible ways, so that future generations of American citizens may be inspired to live according to the example and precepts of Washington's exalted life and character, and thus perpetuate the American Republic, and be it further

RESOLVED, That this resolution be incorporated in the official proceedings of this meeting and that a copy thereof be transmitted to the George Washington Bicentennial Commission, Washington, D. C.

E. M. Bailey: Before turning the meeting over to the President-elect I want to express my gratitude to the members of the committees and to everybody for the splendid cooperation that has been accorded me during this meeting and throughout the year. It is my great pleasure to turn the chair over to Dr. Haskins.

H. D. Haskins: Mr. President and friends of A.O.A.C. I am not going to attempt to make a speech. I simply want to observe that I am deeply grateful for the honor that has come to me and, through me, to the institution which I represent in Massachusetts. Frankly, I should feel all at sea at this time if I were not sure of the very complete organization that has been built up here through the untiring efforts of our good friend, Dr. Skinner, and his associates. I just want to add that if I am fortunate

enough during the next year to preside over your deliberations with one-half the grace, the dignity and the effectiveness of my predecessor, my good friend Dr. Bailey, I shall feel that I have done a creditable job. I am not unmindful of the responsibilities that go with the position and I want to say that I shall need your sympathy and support, and I hope you will bear with me in any mistakes or omissions that I may make owing to my youth and inexperience. I thank you.

CONTRIBUTED PAPERS

DIRECT DETERMINATION OF AVAILABLE CARBON DIOXIDE[1] IN BAKING POWDER

By MAYNE R. COE (Food Research Division, Bureau of Chemistry and Soils, Washington, D. C.)

The well-known gasometric method[2] for estimating the available carbon dioxide in baking powder requires two determinations; namely, those for total and residual carbon dioxide. By subtracting the latter result from the former, the available carbon dioxide is obtained. Although this is a definite step in advance of the official gravimetric method with the Knorr apparatus, the fact that two determinations are necessary for the final result leaves room for still further improvement.

The writer proposes the following new method, which uses the same apparatus and requires only one determination:

Run 25 cc. of 5 per cent ammonium sulfate solution into the 250 cc. decomposition flask containing 1.7 grams of baking powder. Heat the flask and contents at boiling temperature until all gas is evolved and then cool to room temperature. When equilibrium is established, which generally takes about 15 minutes, read the amount of available carbon dioxide on the graduated tube, and make the necessary corrections for temperature and pressure.

Total carbon dioxide also may be determined in a few minutes with the same sample without detaching the flask from the apparatus in the following manner:

Fill the buret containing a few cubic centimeters of $(NH_4)_2SO_4$ with sulfuric acid (1+5) and run 25 cc. into the flask. Heat the flask almost to boiling, allow to cool as before, and take the reading. Subtract a number corresponding to the number of cubic centimeters of acid used from the number of cubic centimeters found, and make corrections for temperature and pressure. This result gives the total carbon dioxide. Obtain the result for residual CO_2 by subtracting the result for available CO_2 from that for the total.

Ammonium sulfate solution is used as a reagent for three reasons: (1) a satisfactory evolution of carbon dioxide is obtained from all varieties of baking powder; (2) it acts as a protein coagulant for powders containing egg albumin, thus serving as a foam destroyer; (3) if distilled water is used with phosphate powders and those containing egg albumin, low results are obtained; but with 5 per cent ammonium sulfate solution the same results are obtained as with the geometric method; (4) if the total CO_2 value is desirable, the acid reagent may be used to evolve the residual CO_2 remaining in an ammonium sulfate solution.

Details of the method are as follows:

[1] Contribution No. 97 from Food Research Division. Part of the work was done in the Cattle Food Laboratory, Food and Drug Adm.

[2] *Methods of Analysis, A. O. A. C.*, 1925, 305; *This Journal*, 6, 453 (1923).

REAGENTS

(a) *Ammonium sulfate solution:* 5 grams of ammonium sulfate made up to 100 cc. with water.

(b) *Sulfuric acid solution* (1+5).

(c) *Displacement solution.* Dissolve 100 grams of sodium chloride or sodium sulfate crystals in 350 cc. of water. Add approximately 1 gram of sodium bicarbonate and 2 cc. of methyl orange indicator and then sufficient of the sulfuric acid (1+5) to make just acid (a decided pink color). Stir until all carbon dioxide is removed. (This solution is used in the gas measuring tube and leveling bulb, and it seldom needs to be replaced.)

APPARATUS

The apparatus used has been described.[1]

DETERMINATION

Place 1.7 grams of baking powder in the dry decomposition flash and connect immediately with the gasometric apparatus. Open the T-tube stopcock, and by means of the leveling bulb bring the displacement solution to the 25 cc. graduation above the zero mark. Allow the apparatus to stand 1–2 minutes to equalize the temperature and pressure within the apparatus with those of the room. Close the stopcock, lower the leveling bulb somewhat to reduce the pressure within the apparatus, and slowly run into the decomposition flask from the buret 25 cc. of 5 per cent ammonium sulfate solution. Then heat gradually to boiling. (Heat must not be applied for too long a period, otherwise the pressure will force the displacement liquid into the leveling bulb to a point where the gas will bubble through the liquid and spoil the determination. This feature may be controlled by marking the danger point on the leveling bulb.) Make sure that all gas is not driven out of the decomposition flask, cool slightly, and heat again. Keep the displacement solution in the leveling bulb at all times during the decomposition at a lower level than that in the gas measuring tube to prevent the liberated carbon dioxide from escaping. Vigorously agitate the decomposition flask to release any occluded gas, allow it to stand about a minute and then cool to room temperature by placing in a beaker of water. (The flask is at equilibrium when the reading of a thermometer immersed in the beaker of water corresponds to room temperature and the volume of carbon dioxide remains constant.) Remove the beaker and wipe off adhering drops from the flask. Equalize the pressure in the measuring tube by means of the leveling bulb, and read the volume of gas evolved. Multiply the factor in the table[2] corresponding to the barometric reading and the room temperature by the number of cc. of CO_2 found and divide by 10 to obtain the percentage of available carbon dioxide by weight.

When the total CO_2 value is desired, keep the T stopcock closed and the leveling bulb at the lower level and then fill the buret containing a few cubic centimeters of ammonium sulfate with 1+5 sulfuric acid solution. Run 25 cc. of acid into the flask, which is still attached to the apparatus. Heat almost to boiling and then allow to cool until equilibrium is established. Note the reading. Subtract 25 cc. (corresponding to the number of cc. of acid used) from the final reading and make corrections for temperature and pressure. Obtain the result for residual CO_2 by subtracting the result for the available CO_2 from the total CO_2 found previously.

Those who are familiar with this determination will observe that the results obtained by the proposed method check as closely as would duplicates obtained by the gasometric method.

[1] *This Journal*, 6, 453 (1923).
[2] *Ibid.*, 10, 37 (1927).

Results for available carbon dioxide in baking powder by the gasometric method and the proposed method.

SAMPLE NUMBER	PROPOSED METHOD	GASOMETRIC METHOD	REMARKS
	per cent	*per cent*	
F.C.4646-A	14.1	13.9	Mixed phosphate and alum powder without egg albumin.
F.C.4648-A	13.2	13.3	Mixed phosphate and alum powder with egg albumin.
F.C.4649-A	13.8	14.0	Mixed phosphate and alum powder with egg albumin.
F.C.4650-A	14.4	14.3	Pure phosphate powder without egg albumin.
F.C.4653-A	12.0	12.1	Pure phosphate powder with egg albumin.
F.C.3394-A	13.4	13.4	Pure phosphate powder without egg albumin.
F.C.4652-A	12.6	12.3	Tartrate powder.
F.C.3593-A	13.1	12 9	Tartrate powder.
F.C.3598-A	12.7	12.5	Tartrate powder.
F.C.3487-A	12.6	12.4	Tartrate powder.
Average	13.2	13.1	

SUMMARY AND CONCLUSIONS

(1) The method described provides a direct means of estimating the available CO_2 in a baking powder and also a method for estimating the total CO_2 with the same sample.

(2) The residual CO_2 may be obtained by subtracting the result for available CO_2 from that for total carbon dioxide.

(3) The time necessary to make a determination is much shortened.

(4) Laboratories already equipped with the apparatus for the gasometric determination may use the procedure described without additional expense.

(5) The limiting factors, such as equilibrium and vapor pressure, control the accuracy of the method.

A FEW IMPRESSIONS OF AGRICULTURAL CHEMISTRY IN FOREIGN COUNTRIES[1]

By C. A. BROWNE (Bureau of Chemistry and Soils, Washington, D. C.)

It has been suggested that I speak to you for a few minutes this afternoon upon some of my impressions of agricultural chemistry obtained during a sojourn of sixteen months in various foreign countries. The main purpose of my trip abroad was to study some of the numerous applications of chemistry to agriculture, and chemical analysis, which informs us about the composition of our soils, fertilizers, insecticides, crops, cattle

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists, held at Washington, D. C., October, 1930.

feeds, milk and other agricultural products, is certainly one of the most important of these applications. There has unfortunately been a decline in the analytical training which students receive today at college as compared with that of a generation or more ago. The old fundamental courses in qualitative and quantitative analysis, based upon such works as those of Fresenius, are now encroached upon by other studies. The result is that you hear everywhere both in the United States and in Europe the complaint that good analysts are becoming more and more difficult to find. Liebig, the founder of modern agricultural chemistry, made analysis the foundation of all of his chemical training and I am old fashioned enough to believe that his method is still the only correct one.

One of the greatest pleasures of my trip was a visit to the old laboratory of Liebig at Giessen, where one can follow the developments of analytical chemistry under this great master, as improvements were introduced. In the oldest of these laboratories there are a number of primitive furnaces for the accommodation of retorts, evaporating dishes and other apparatus. As the number of students increased, another furnace or chemical hearth was built in the middle of this compartment. Tall portable chimneys were used to conduct smoke and fumes to the upper part of the room where they escaped through the open doors and windows. This arrangement was wholly inadequate for proper ventilation, and Liebig has left an account for the year 1832 of the miserable conditions of vapors, dust and drafts under which his students had to work. In this building one can see the old sample room, preparing room, balance room, library and other compartments with exhibits of the combustion furnaces, absorption bulbs, condensers and other historic apparatus which Liebig invented and employed with such results that the whole course of our civilization was changed. The science of modern agricultural chemistry was laid here, and I tried to visualize during my visit the long line of famous students from all parts of the world (E. N. Horsford and C. M. Wetherill of the United States among the number) who went out from this place to spread the message of their teacher.

In the old Ashmolean Museum at Oxford University I saw historic analytical apparatus of another famous early worker in the field of our science. I refer to Sir Humphry Davy whose "Agricultural Chemistry" was the first noteworthy book upon the subject in the English language. Davy devised a very ingenious piece of apparatus, exhibited in the old Ashmolean, for determining the lime content of soils. The CO_2, produced by the action of acid upon a weighed sample of the soil, was conducted into a bladder immersed in water. The water, displaced by the expansion of the bladder, was measured and from its volume was calculated the lime content of the soil.

In the Conservatoire des Arts et Metiers in Paris I saw even earlier analytical apparatus of far greater historic value and importance. These

were the original balance, pneumatic trough, gasometer, thermometer, calorimeter and other contrivances which Lavoisier employed in his quantitative studies of fermentation, respiration, synthesis of water, combustion of oil and other subjects by which he was enabled to lay the foundations of modern chemistry. The visitor is impressed by the size, solidity and elegant construction of this early apparatus which has hardly been surpassed for beautiful workmanship by anything of a later date.

Time does not permit me to dwell longer upon historic matters, and I shall therefore pass on to a consideration of certain developments of present day interest.

The greatest contribution that has been made in the field of chemical analysis during the present century is that of Professor Pregl at the University of Graz in Austria upon micro-methods. Pregl's apparatus for microchemical analysis was employed in every first class laboratory of Europe which I visited. Improved balances, combustion furnaces, burets, filtering tubes, Kjeldahl apparatus, electrodes, sublimators, and many other devices for micro determinations were exhibited at the recent Achema Exposition held at Frankfort in Germany which I attended last June. The latest microchemical balances are provided with counterpoised dampening cylinders of sheet aluminum which eliminate swinging of the beam so that when the pan is released at the final weighing the pointer stops immediately over the scale division which indicates through a magnifier the hundredth and thousandth part of a milligram. These balances are so extremely delicate that they require special care in mounting and manipulation. All sources of extraneous vibration and all disturbances due to draughts, inequalities of temperature and other causes must be prevented. One chemist informed me that he never lifted a hand to one side of the balance without making a corresponding movement with the other hand to the opposite side in order that the influence of radiation might be equalized.

The methods of micro-analysis are of the greatest importance in agricultural chemical research in view of the growing realization of the significance of minute quantities of certain elements in plant and animal nutrition, as demonstrated by Bertrand at the Pasteur Institute in Paris, by McHargue at the Kentucky Experiment Station, by Brenchley at Rothamsted in England and by other workers. In the determination of minute quantities of the essential constitutents of agricultural products the chemist is greatly indebted to the improvements in analytical technic which have been accomplished by Pregl and others of his school. Those who are interested in this subject are referred to the recent collaborative Festschrift, entitled "Mikrochemie," which was issued recently in celebration of Pregl's sixtieth birthday.

Another important movement of American origin in analytical chemistry which is winning great headway in Europe, more especially in Ger-

many, is the normalization of apparatus. In my visit to the last Achema Exposition the first object which attracted my attention was a cork suspended from the ceiling to which forty strings were attached leading to as many bottles, flasks, separatory funnels and other laboratory utensils that required the employment of this useful article. Beneath this cork was the following placard in large letters:

Die Normung hast du hier erfasst.
Ein Kork auf 40 Sachen passt.

As one who had wasted many precious hours in hunting through laboratory drawers for a cork that would fit, this exhibit immediately appealed to me, as it did to every visiting chemist. Numerous other displays illustrated the great benefits which are to be derived from a simplification and unification in the construction of chemical apparatus. The making of all cover glasses of the same convexity to assist in stacking is another illustration. The movement has for its aim the avoidance of multiplicity in the dimensions for tubing, stop cocks, connections, and other parts of apparatus with the securing of the greatest possible degree of interchangeableness. This is only a part of the more general normalization or rationalization movement that has permeated all German industries since the close of the war as a result of the similar reform, first inaugurated in the United States by the Bureau of Standards, under the leadership of Herbert Hoover when he was Secretary of Commerce in 1921, to whom full credit is given in the extensive German literature upon the subject.

The same conception of normalization is also being most thoroughly carried out in Germany in the erection of new laboratories, where plumbing, hoods and desks, ventilating equipment and all other devices are brought into alignment with the ideas of simplification and convenience as based upon the accumulated experience of leading chemists. I saw several new laboratories that had been constructed along these lines, the most complete which came to my notice being the newly erected research laboratory of E. Merck and Company at Darmstadt. The plumbing is all of the open type, and the various pipes for water, gas, steam, compressed air, vacuum, etc., are painted a different color from point of origin in the basement to the outlet cock in each laboratory. The arrangements for balance rooms, titration tables, desks, etc., are in accordance with the best results of experience. It was also my privilege to visit the new research laboratories of the German Potash Syndicate at Lichterfelde, near Berlin, and of the Prussian Hygienic Institute at Landsberg, which are also models of excellence.

Of other recently constructed laboratories which impressed me with their fine equipment I should mention that of the Colonial Institute in Amsterdam, the most palatial of any which I saw; the beautiful new agricultural laboratory of the University of Montpellier in the south of France

and the magnificent food research laboratories of J. Lyons and Company at Cadby Hall, London. I regret that there is not time to give an account of some of the things which I observed at these places.

It must be remarked, however, that owing to the very depressed economic conditions which exist in most European countries as a result of the World War many of the university laboratories are in a state of great privation. I was surprised upon revisiting the old laboratory of Tollens at the Agricultural Institute of Göttingen to find the desks, hoods, and other equipment to be almost the same as when I worked there thirty years ago. There was no money available for improvements. It was the same at other research laboratories which I visited. At Münster, in the laboratory of the late Professor König, whose works upon the chemical analysis of agricultural products are universally known, I was shown a laboratory-made potentiometer for determining the *p*H of soils. Funds were not available for purchasing the expensive 600 mark instrument, and students had to construct their own apparatus. As a result of this discipline I believe German students are being much more thoroughly grounded in the fundamentals of laboratory technic than are the students of our own country. Necessity is the mother of invention, and privation sometimes acts as a stimulus instead of a check.

One thing which impressed me particularly during my visit to Germany was the fact that notwithstanding the present period of extreme industrial and agricultural depression the large corporations, such as the I. G. Dye Industry and the Potash Syndicate, were maintaining their research laboratories at the highest degree of efficiency. A director of one of these organizations informed me that their research laboratories were the very last thing that would be closed as a result of hard times. Their success was due to research; many years had been required to build up their research staff, which was therefore not to be broken up except in the last extremity; furthermore in just such times of depression there was the greatest need of research for devising new economies and the means of overcoming present difficulties.

The largest and most completely equipped agricultural experiment station that I saw during my trip was that of the I. G. Dye Industry at Limburgerhof, near Ludwigshafen. The greatest range of investigations is conducted here, not only upon the crops of Europe but upon the sugar cane, rice, cotton, banana and other plants of the tropical and semitropical world. The effects of different fertilizers, of different colored light, of CO_2 concentration and of many other factors which influence the growth of crops are investigated here. The composition of rain water, the curing of tobacco, methods of irrigation, lysimeter studies, the effects of paper mulching, the production of artificial stable manure, problems of plant metabolism, the effects of fertilizers upon the vitamin content of crops and other subjects too numerous to mention are also being studied. The vege-

tation houses at Limburgerhof are the largest which I have seen; they contain 5000 Mitscherlich pots in which experiments are conducted upon all types of crops and soils.

The newly established agricultural research station of the Imperial Chemical Industries, Ltd., which I visited at Jealott's Hill in England, is another illustration of how chemical industries upon the other side of the Atlantic are interesting themselves more and more in agricultural investigations. Intensive grassland management, the production of concentrated feeds by the drying of nitrogenous grasses, experiments in plant and animal nutrition and numerous other topics are being investigated at this station. A certain amount of prejudice has had to be overcome in the minds of some farmers who regard the results of agricultural research obtained by industries as biased and in the nature of propaganda. Corporations of well established reputations cannot afford, however, to jeopardize their good names by sending out fictitious results. The entrance of industries into agricultural experimentation I regard as a very propitious sign. The industries benefit by obtaining exact first hand information about the employment of their products whether fertilizers, insecticides or cattle feeds. Agricultural science and practice also benefit, as the industries are financially able to conduct expensive researches which are beyond the means of the older type of university experiment station.

The Chambers of Agriculture constitute a third class of agricultural experiment station which I observed in Germany. These are located in each province, and they render most valuable practical services, such as analyzing soils, fertilizers, and cattle feeds; investigating plant and animal diseases and giving scientific advice through staffs of highly trained specialists. A nominal charge is made according to the character of the work, but this charge is very low in comparison with the great value of the service rendered. You can form some idea of the volume of work done at these chambers when I tell you that in the one at Stettin, which I visited, they made 54,000 *p*H determinations of soils in Pomerania for 1929. Similar attention is given to the *p*H of soils in the other provinces of Germany, and the farmers who make use of the various chambers of agriculture are advised concerning the crops for which their fields are best adapted. They are shown also how to correlate the natural vegetation of their fields with soil reaction.

In the Chamber of Agriculture at Stettin, where they have over 3000 Mitscherlich pot experiments, I was puzzled to observe the tests which were being conducted upon a considerable number of common weeds. It was then explained to me that weeds are a most valuable indicator of soil reaction. The weed *Spergula arvensis*, for example, thrives best in an acid soil with a *p*H range of 3 to 5, while the weed *Sinapis arvenis* prefers an alkaline soil with a *p*H range of 6.5 to 8. Tables showing the *p*H preferences of the common weeds and also of all the various crops of Germany

are distributed by the Chambers of Agriculture so that the farmer, if he observes sorrel, an acid-loving weed, growing abundantly upon a particular field, will know that its soil is not adapted to the cultivation of the sugar beet, barley or lucern, which prefer a slightly alkaline soil of *p*H 7 to 8, but that it will support with much better chances of success potatoes, timothy, lupines and oats, which prefer an acid soil of *p*H 5 to 6.

In correlations of this kind the German farmer appears to be better informed than his American brother. Dr. Spengler of the Sugar Institute of Berlin told me that during his recent tour of America he observed some of our farmers trying to grow sugar beets in fields where the common sorrel was plentiful, a thing which any German farmer would know ought not to be done.

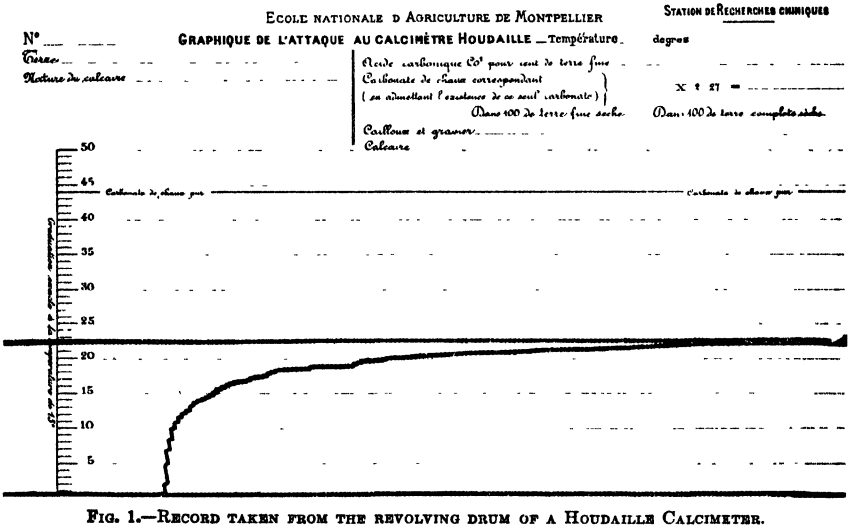


FIG. 1.—RECORD TAKEN FROM THE REVOLVING DRUM OF A HOUDAILLE CALCIMETER.

Another interesting example of the correlations between crop production and soil characteristics is afforded by the work being done in Europe upon the study of the effect of deficiencies of nitrogen, phosphorus, potash, calcium, magnesium, etc., in the soil upon abnormalities in the shape, color and other appearances of the leaf, a deficiency of any particular element producing a characteristic malformation or abnormality. This particular branch of agricultural chemistry, known as foliary diagnostics, has been especially developed by Mr. Wallace at Long Ashton, England, in the case of fruit trees, and by Dr. Krüger, director of the Experiment Station at Bernburg, Germany, in the case of the sugar beet, potato and other field crops, both of whom I had the pleasure of visiting.

Professors Lagatu and Maume, at Montpellier in France, have also given much attention to foliary diagnostics, but these authorities lay greater stress upon the abnormalities produced in the composition of the

ash of the leaves than upon the abnormalities in physical appearance. Time is lacking to discuss some very interesting deductions of their work, more especially with regard to Liebig's "Law of the Minimum." In my talks with Professor Lagatu he pointed out the injurious effects of improperly balanced fertilizers, which may depress the yield of crops to such an extent that less is obtained than upon the check plots where no fertilizer at all was used. These apparent contradictions have caused Professor Lagatu to suggest the adoption of the phrase "Law of the Optimum" in place of the term "Law of the Minimum."

Professor Maume showed me an interesting apparatus, called the Houdaille Calcimeter, for measuring the speed or rate of attack with which lime is decomposed in the soil, this factor having an important bearing upon the fertility of certain French soils. A weighed amount of soil is treated in the apparatus with a measured volume of dilute hydrochloric acid. The pressure of the evolved CO_2 moves a pointer against a diagram upon a revolving drum and traces a curve which records both the amount of lime in the soil and the speed with which it is decomposed. (See Fig. 1.)

As you all know, there has been a vast amount of discussion in Germany with respect to the relative advantages of the Mitscherlich, Neubauer and other methods for determining the fertilizer needs of soils. The Mitscherlich method necessitates waiting until the maturity of the plant, which is grown in a rather expensive specially constructed pot. The Neubauer method is based upon the ash analysis of rye seedlings, grown in an ordinary glass crystallizing dish, and requires only 18 days for completion of the test. Niklas, experimenting at Weihenstephan in Germany with Aspergillus Niger and the Azotobacter, has developed other processes which reduce the time of testing to less than a week. I noticed a tendency in a number of European laboratories to depart entirely from such biological methods and to return to methods of soil extraction by means of special solvents. Professor Wrangell, whom I visited at Hohenheim, Germany, has adopted the still more fundamental principle of studying the deficiencies in composition of the soil solution itself, which she analyzes by means of the improved micro-methods.

Leaving now the subject of soils, which is demanding the major part of the attention of agricultural chemists in the various laboratories which I visited, I should like to refer very briefly to some of the work being done upon foods and drugs. Here again, and especially in Germany, there is the greatest scientific activity, all the latest discoveries of science being applied to the solution of both research and regulatory problems. Ultraviolet light is employed extensively in all sorts of ways. In the laboratory of E. Merck and Company at Darmstadt I was shown two specimens reputed to be Chinese rhubarb. The genuine sample did not fluoresce in ultra-violet light while the adulterated sample fluoresced very strongly. Novocaine fluoresces very strongly while cocaine does not. Numerous

other applications of a similar character were made in other laboratories which I visited. Ultra-violet light was also employed in examining the contents of the stomach in post mortem cases.

The photoelectric cell is another new appliance which I found to be extensively used in many European laboratories for making comparisons in colorimeters and photometers and also for plotting the absorption spectra of liquids. For the latter purpose the photographic film of the spectrum is passed between the photoelectric cell and a beam of light; the deviations upon a galvanometer, produced by the varying intensities of the absorption bands, are noted for each part of the spectrum, and the readings are then plotted upon a curve.

In the field of sugar analysis I noticed many interesting developments during the course of my travels. Among these was the use of the 20 gram normal weight saccharimeter in the sugar factories of Egypt. This, of course, is not a new procedure. A decimal system normal weight was used over fifty years ago upon the old Wild polaristrobometer. A movement to adopt an international 20 gram normal weight in saccharimetry was advocated some thirty years ago by Pellet and Sidersky in France, and just after the war I attempted with several English and American colleagues to revive this proposal. Some of the repercussions of the debates upon this question may be found in the back proceedings of our association. The influence of established custom however, was too strong, and the proposed reform, as in previous cases when it had been recommended, was not generally accepted although most manufacturers of saccharimeters are now making 20 gram normal weight instruments. In the sugar factories of Egypt this type of saccharimeter has been in general use for some time, and the chemists whom I met there expressed the opinion that the advantages of the 20 gram normal weight, as regards simplicity, convenience and economy of time, are so great that any one who has once made use of it will never employ anything else.

Of the various natural sugar-containing products, honey is probably receiving more attention from European chemists than any other commodity, and during my travels I had an excellent opportunity to confer with honey experts in England, Germany, France and Switzerland. They are laying much stress at present in Germany upon the enzymes of honey, more particularly the diastatic enzymes, a deficiency in these being regarded as an index of injury to the honey by overheating. Professor Ambruster, whom I visited at Dahlem near Berlin, is one of the leaders in the researches upon honey enzymes.

Another phase of honey research which has received much attention in European laboratories is the detection of added invert sugar as an adulterant. The color tests ordinarily used for this purpose depend upon the presence of oxymethylfurfural, a decomposition product of fructose produced by the high temperature at which the sugar sirup is inverted. A re-

agent which I proposed for this purpose in 1907 was a solution of anilin in glacial acetic acid. It was adopted by our association, although one of the referees later modified the test by substituting anilin chloride. The effective ingredient, however, whatever salt be employed, is the anilin that I originally proposed in preference to certain other coloring reagents that I rejected because of their supersensitiveness.

Shortly after the publication of my method, Fiehe in Germany proposed the use of Seliwanoff's reagent, a solution of resorcin in hydrochloric acid, as a means of detecting added invert sugar in honey. This is much more sensitive than anilin as a color test, but great care is needed in its interpretation. A heated honey may give a faint pink or rose coloration which is frequently mistaken for the vivid cherry red characteristic of the Fiehe test. This reaction has led to frequent errors upon the part of analysts in condemning pure honeys as adulterated. Cardenas and Moreno[1] pointed out in 1921 that many genuine Cuban honeys that fail to react with Browne's anilin reagent give a positive Fiehe test. Fiehe, however, has shown that this is due to a misinterpretation of his color reactions.

A later illustration of the mistakes resulting from misinterpretation of the Fiehe test occurred just before my departure for Europe. A large shipment of American honey to France was condemned by the French Government because their chemist reported the presence of added invert sugar upon the basis of the Fiehe test. This chemist had written a book upon honey analysis in which he fully described the Fiehe test with numerous colored plates and so presumably he was qualified to render an opinion. A sample of this honey examined at our Bureau gave, however, only a pink coloration with nothing of the intensity characteristic of the true Fiehe reaction. The Browne anilin test was also negative. For a final decision in the matter, a sample of the condemned honey was then sent to Dr. Fiehe, who reported that the French chemist had misinterpreted the color of the reaction and that there was no adulteration with invert sugar, the slight pink color which was noted being probably due to an overheating of the honey. This incident illustrates the extreme care which regulatory chemists should employ in interpreting the Fiehe test.

The 30th of last June, the day of Dr. Wiley's death, I spent at the Prussian Hygienic Institute in Landsberg discussing these and numerous other problems of honey analysis with Dr. Fiehe himself. It was interesting for me to note that at the time of my visit he was extracting the oxymethylfurfural from suspected honeys by means of a percolating tube, using the same process as that employed by Nelson in his work at our Bureau. You may be interested to know that Fiehe has gone a step further than we have in this development in that he determines the oxymethylfurfural, after its

[1] "Las Mieles de Abeja de Cuba," Julio de Cardenas and Eduardo Moreno. Bulletin of the Agricultural Laboratory of the Department of Agriculture, Commerce and Labor, Havana, Cuba.

complete extraction from the honey, by precipitating with phloroglucinol and weighing the resultant phloroglucide. This quantitative improvement in the method should eliminate completely the mistakes which result from a misinterpretation of the color reaction. I regret that the details of this quantitative procedure[1] arrived too late to be included in the next edition of our published methods.

The second day after my visit with Dr. Fiehe I spent at Dresden visiting the recent Public Health Exposition of that city, the finest exhibition of the kind which it has ever been my fortune to attend. A great deal of attention was given to food chemistry and food control. The development of pure food legislation in Germany was illustrated by wall charts, pictures and books. There was a map showing the location of the Food Control Stations of Germany. In 1880 there were only seven of these, in 1890 there were twenty-seven, in 1905 there were one hundred and fourteen, in 1910 there were one hundred and fifty-four, and in 1930 there are one hundred and forty-five. The decrease since 1910 is due to the depression of activities as a result of the World War. Numerous photographs of typical food control laboratories were shown, and there were many individual exhibits relating to special topics such as poisonous metals, saccharin, food colors, milk products, beverages, etc. There were also extensive exhibits of the analytical apparatus employed in food control work. Methods of food inspection, sanitation, fumigation for the destruction of noxious insects, and countless other applications of chemistry to public health matters were also illustrated at this exposition.

In the matter of scientific and industrial museums and expositions the European countries are generally far in advance of the United States, and I was led to reflect that this may be one explanation of their greater productivity in certain fields of research. We excel the nations upon the other side of the Atlantic in many subjects, such for example as the sanitation of our cities and homes, the protection of food against dirt and infection in our public markets, the introduction of the refrigerating machine as a household utensil, the more highly developed technic of our canning, preserving and packing house industries, and the more general application of machinery in the production, transportation and utilization of our agricultural products.

On the other hand I would say as a general result of my observations, during the past one and a half years, that in such agricultural investigations as those concerning the lesser studied elements essential to plant growth, the significance of humus, crop rotation, pasture land management, composting, pot experimentation, foliary diagnostics, green house culture, intensive farming and many of the so-called fundamental lines of research, we are behind the European nations. This is also certainly true,

[1] "Bestimmung des Oxymethylfurfurols" by J. Fiehe and W. Kordatzké, *Z. Untersuch. Lebensm.* 56, 490 (1928).

as it has been for many years, of methods for the chemical analysis of foods and other agricultural products. We are not laggards in this field by any means, but we have as yet made no outstanding contributions corresponding to the great work of Pregl and we have as yet published no books corresponding to the colossal volumes of König upon Agricultural Analysis, of Abderhalden upon Biochemical Analysis or of Willstätter upon Enzymes. We are still, however, a young people, and the future is full of hope.

Perhaps the chief pleasure of my trip was the meeting in foreign lands of many young agricultural investigators who had studied at some of our American colleges and experiment stations. Scarcely less was the pleasure of meeting various American students who were conducting agricultural research work in some of the institutions of the Old World. This interchange of students should be encouraged. The agricultural scientists of the New and Old Worlds have much to learn from each other, and if I were a young man in search of the best instruction in agricultural chemistry I should unhesitatingly go again to Europe for at least a part of my training.

THE DETERMINATION OF STARCH IN FLOUR BY DIASTASE-ACID HYDROLYSIS*

By B. G. Hartmann and F. Hillig (Food Control Laboratory,[1] Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

In the method herein described complete gelatinization of starch is assured before diastase hydrolysis is applied. In the official method[2] 2 hours is required for the starch conversion, whereas 20 minutes is ample time for the conversion procedure proposed.

The pepsin digestion was introduced because it has been shown by the writers[3] that the application of the enzyme aids in the conversion of the starch content of materials high in proteins, and in the present investigation it has been proved that peptic digestion materially increases the starch yield. In the pepsin digestion an acidity adjustment corresponding to 5 cc. of normal hydrochloric acid per 100 cc. of substrate is made. After the digestion the acid in the mixture is carefully neutralized with alkali, slightly acidified with hydrochloric acid, and finally treated with calcium carbonate for the purpose of assuring a practically neutral condition for the proper functioning of the diastase. The total working time required for the peptic-diastatic digestion is about 3 hours.

* Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1930.

[1] W. B. White, Chief, Food Control.

[2] *Methods of Analysis, A. O. A. C.*, 1925, 119.

[3] *This Journal*, 9, 482 (1926).

The official method has been further modified in regard to the removal from the flour of the sugars and free fat. The material treated by the modified procedure is practically free of sugars, and it is believed to be more reliable and less arduous than that prescribed in the official method.

The procedure outlined is time consuming, but it is easily carried out and with ordinary care should give satisfactory results even in the hands of analysts inexperienced with it. Special precaution should be observed, however, in the sugar determination owing to the small quantity of dextrose which is present in the malt extract aliquot. It is recommended that the Gooch crucibles, empty and charged, be kept in the desiccator 15 minutes before weighing. No trouble was experienced in obtaining duplicate determinations of cuprous oxide which agree within a few tenths milligrams.

THE METHOD

REMOVAL OF SUGARS AND FREE FAT

Accurately weigh 2.5–3.5 grams of the flour, ground to pass a 40-mesh sieve, into a *dry* 16 ounce tincture bottle. Add 100 cc. of ether and shake 5 minutes, add 100 cc. of 95 per cent alcohol and shake 5 minutes and then add 50 cc. of water and shake 5 minutes. Wash the sides of the bottle with 70 per cent alcohol (70 cc. of 95 per cent alcohol made to 100 cc. with water) and centrifuge 10 minutes at about 900 r.p.m. Decant the liquid, and to the residue add 150 cc. of 70 per cent alcohol; shake thoroughly, wash the sides of the bottle with 70 per cent alcohol, and centrifuge. Repeat the centrifuging operation, pour off the liquid, and drain by inverting the bottle several minutes.

CONVERSION OF THE STARCH

Transfer the residue in the bottle to a 400 cc. beaker with about 150 cc. of water, removing with a curved policeman any starch that may adhere to the sides of the bottle. Now add 7.5 cc. of normal HCl to the contents of the beaker; bring to a boil, stirring constantly; cover with a watch glass; and place on a briskly boiling steam bath for 2 hours. Allow the mixture to cool to about 40°C., add 10 cc. of a 1 per cent pepsin solution, mix thoroughly, and allow to stand overnight at a temperature between 30° and 40°C. The next morning add normal NaOH to faint alkalinity (phenolphthalein) and come back immediately with normal HCl, adding three drops of the acid in excess. Add 0.2 gram of calcium carbonate and heat to boiling, stirring constantly. Allow the mixture to cool to 70°C. and place in a water bath adjusted to a temperature of about 70°C. Add 10 cc. of malt extract solution prepared by digesting 20 grams of ground malt with 200 cc. of water for 2 hours and filtering. (The addition of the 10 cc. of malt extract solution will reduce the temperature of the digestion mixture to about 65°C.) Maintain this temperature for 10 minutes, stirring the mixture frequently. Now add 10 cc. more of the malt solution and digest an additional 5 minutes. Bring the mixture to boiling and boil 5 minutes, stirring constantly. Cool to 70°C. and again digest 5 minutes with 10 cc. of the malt solution. Heat to boiling, boil 5 minutes, transfer to a 300 cc. volumetric flask, cool to 20°C., make to mark with water, shake, and filter through a folded filter. Transfer 200 cc. of the filtrate to a 500 cc. volumetric flask, add 20 cc. of dilute hydrochloric acid (2+1), and heat in a boiling water bath for 2½ hours. Cool to 20°C., make to mark with water, shake, and filter. Transfer 40 cc. of the filtrate to a 400 cc. beaker and neutralize with 10 per cent sodium hydroxide (phenolphthalein). Make slightly acid with normal HCl and adjust to a volume of 50 cc. with water. Determine dex-

trose by the Munson-Walker method.[1] Run a blank on the malt extract solution in the same manner as in the determination.

CALCULATION

$$S = \frac{1.6875\,(a-b)}{X}.$$

S = percentage of starch.
a = dextrose in aliquot of sample (mg.).
b = dextrose in aliquot of the blank (mg.).
X = sample taken (grams).

The various steps in the procedure were carefully investigated in order to assure that the claims made for them are justified. The procedure for the removal of sugars was tested by extracting 8 grams of wheat flour with

TABLE 1.

Effect of pepsin digestion on the yield of starch.

MATERIAL	DEXTROSE IN ALIQUOT		DIFFERENCE
	WITH PEPSIN	WITHOUT PEPSIN	
	mg.	*mg.*	*per cent*
Wheat starch	148.9	145.1	
	149.1	145.4	2.8
	Av. 149.0	Av. 145.3	
Cornstarch	152.1	149.5	
	151.7	150.2	1.5
	Av. 151.9	Av. 149.9	

ether-alcohol, as described in the proposed method, and subjecting the residue in the centrifuge bottle to two additional extractions of 100 cc. each of the 70 per cent alcohol. A sugar determination on the combined additional extractions did not yield a trace of cuprous oxide.

In order to show the effect which the pepsin digestion has upon the yield of starch, dextrose determinations were made on wheat starch and cornstarch, with and without the use of pepsin. The results are shown in Table 1.

From the results shown in Table 1 it is apparent that if a pepsin digestion is made before hydrolysis is applied, the yield of starch will be materially increased. That the use of pepsin in the procedure is not responsible for the increase in starch is shown in Table 2. The pepsin merely digests proteins, thereby making the starch accessible to diastase hydrolysis. Thirty milligrams of glutenine with and without pepsin treatment was subjected to malt and acid hydrolysis, as described in the method, and the resulting dextrose was determined.

[1] *Methods of Analysis, A. O. A. C.*, 1925, 190.

TABLE 2.

Effect of cleavage bodies of glutenin on the dextrose determination.

DEXTROSE IN ALIQUOT	
With Pepsin	Without Pepsin
mg.	*mg.*
43.8	43.4
44.0	43.9
Av. 43.9	Av. 43.8

Apparently no copper reducing substances are produced by the treatment with pepsin.

The method was applied to four commercial starches and a wheat flour.

TABLE 3.

Results expressed in percentage on commercial starches and wheat flour.†*

DETERMINATION	TAPIOCA STARCH	POTATO STARCH	CORN-STARCH	WHEAT STARCH	WHEAT FLOUR
Moisture	12.49	15.37	11.83	11.76	10.63
	12.50	15.41	11.86	11.75	10.67
Average.....	12.50	15.39	11.85	11.76	10.65
Fat (acid hydrolysis)	0.10	0.18	0.43	0.69	2.20
	0.09	0.18	0.44	0.66	2.10
Average.....	0.10	0.18	0.44	0.68	2.15
Protein (N.×5.7)	0.16	0.12	0.36	0.24	10.60
	0.16	0.13	0.38	0.24	10.55
Average.....	0.16	0.13	0.37	0.24	10.58
Ash	0.10	0.24	0.12	0.28	0.52
	0.11	0.24	0.12	0.25	0.54
Average.....	0.11	0.24	0.12	0.27	0.53
Total sugars (As dextrose)	—	—	—	—	1.20
Starch (difference)	87.1	84.1	87.2	87.1	74.9
Starch (by proposed method)					74.5
					74.2
	86.7	84.0	86.9	86.1	74.3
	86.9	84.0	86.5	86.3	74.5
Average.....	86.8	84.0	86.7	86.2	74.4
Undetermined	0.3	0.1	0.5	0.9	0.5

* The starches were not extracted with alcohol-ether.
† This flour was submitted by the Referee on Starch in Flour.

The nature of the undetermined portion was not investigated; very likely it consists of small quantities of crude fiber and other minor ingredients. It was noticed that after digestion with pepsin the tapioca and potato starches yielded solutions which were practically bright, while with the corn and wheat starches and the wheat flour the digestion mixtures showed heavy precipitates.

When applied to gluten, graham and wheat flours and semolina, the diastase hydrolysis came to completion in the first treatments with malt extract solution (15 minutes).

SUMMARY

A modification of the official method for the determination of starch by diastase-acid hydrolysis is described. It is shown that the introduction of a digestion with pepsin increases the starch yield. In the official method the time required for starch conversion by diastase is 2 hours, whereas 20 minutes is ample time for the procedure described; however, the total time is about the same for both methods. A modification of the official procedure for the removal of the sugars from the flour has been introduced.

NEW BOOKS

Physical and Chemical Examination of Paints, Varnishes, Lacquers and Colors. By HENRY A. GARDNER. 5th ed. Distributed by Washington Institute of Paint and Varnish Research, Washington, D. C., 1930.

As stated in the preface, "the rapid advances which have taken place in the paint, varnish and lacquer industry during the last three years" makes a revision of this well known book necessary. While the methods for physical examination and testing go to make up a large part of the volume our readers who are interested in the chemical analysis of paints will find a comprehensive chapter of approximately 70 pages giving details of methods. The methods of the American Society for Testing Materials form "the background of this chapter." As stated in the introduction of this chapter "the author or members of his staff have for many years been members of various sub-committees of Committee D-1" of this Society hence the analyst is assured that details of these methods will contain the interpretation of those most familiar with them. The general subheadings of this chapter are: Sampling, A.S.T.M., Tentative Methods of Routine Analysis of White Linseed Oil Paints, D 215–29, T., Analysis of Single White Pigments, Iron Oxides, Chrome and Blue Pigments, Analysis of Black Pigments and Testing Methods on Miscellaneous Pigments. Cross references to other chapters of the book dealing with paints, of particular interest in connection with the analysis of these products and raw materials, are frequent. References to original sources of information are also given, making these available for those who wish more details. A separate chapter of about 16 pages is devoted to the analysis of Pyroxylin Coatings. The author states in his introduction to this chapter "that no comprehensive scheme of analysis has yet been devised which may be considered suitable for all types of nitrate lacquers." The chapter consists mostly of "Materials to Look for in Pyroxylin Coatings," and "Schemes for Analyses of Lacquers."

Nutrition and Food Chemistry. By B. S. BRONSON. John Wiley and Sons, Inc., New York, N. Y., 1930. Price $3.75.

The author believes, as revealed in his preface, that "probably no field of human knowledge is burdened with a greater mass of accumulated misinformation than that of food and diet," and has offered his contribution "with the hope of lessening the burden in some measure." It is primarily prepared for the classroom but it is the author's hope that it will offer the general reader "something of the foundations of nutrition and its later trends." To those of our readers who have little time to assume the burden of searching original sources of information this volume will be of much help as the references are numerous. The scope of the book is indicated by the following chapter headings: I, Food, and Mechanics of Digestion; II, Chemistry of Digestion; III, Chemical Changes in the Intestine and Absorption; IV and V, Composition of Foodstuffs; VI, Fats and Proteins; VII & VIII, Fate of the Foodstuffs; IX, Protein Requirement; X, Energy Requirement; XI, Inorganic Salts and Acid-Base Balance; XII, Vitamins; XIII, Milk and Milk Products; XIV, Butter, Oleomargarin, Cheese, Condensed Milk and Ice Cream; XV, Eggs and Meat; XVI, Vegetable Foods; XVII, Legumes, Root Crops and Green Vegetables; and XVIII, Fruits. Tables in the appendix giving distribution in foods of vitamins A, B, D, E, and G; iron content of foods; of beef tissues; of spleen, liver and kidney from different animals; and copper content of foods are very extensive.

Introduction to Physiological Chemistry. By MEYER BODANSKY. John Wiley and Sons, Inc., New York, N. Y. 2nd ed. 1930. Price $4.00.

Two new chapters have been added in the revision of this book, "one dealing with the composition of foodstuffs and the other devoted to a brief consideration of the

composition of milk and of certain tissues, including bone, cartilage and muscle". While "laboratory methods and the description of tests have been omitted intentionally," it is believed our readers will find this book helpful in correlating physiological chemistry with their specialties. This is the aim of the author as stated in the preface to the first edition. "The main aspects of physiological chemistry have been developed in relation to recent advances in the science" to quote the author. In the preface to the second edition, the author states in part: "the opportunity of incorporating many of the more recent contributions in physiological chemistry" is taken advantage of in this revision. "In reviewing these contributions he has abstained from conveying an impression of finality and has attempted to show the changing aspects of the subject. The experience of the last few years has proven that even long cherished conceptions and apparently well established facts may at any time undergo drastic revision. The same may be expected in the future. With this subject in such a state of flux and rapid development it is not likely that even a frequently rewritten textbook will quite keep abreast of the times and it is therefore especially desirable that the student of biochemistry should be introduced to the literature as early as possible."

Therefore, the author has included as abundant a bibliography as space permits. A subject and author index are included.

A Comprehensive Treatise on Inorganic and Theoretical Chemistry. Vol. X. Sulphur and Selenium. By J. W. MELLOR. Longmans, Green & Company, New York, 1930. 958 pp. Price $20.00.

The appearance of this volume will be welcomed by those familiar with the work of this author. His books need no comments other than press notice that they are completed.

An Introduction to the Chemistry of Plant Products. Volume II. Metabolic Processes. 2nd ed. By PAUL HAAS and T. G. HILL. Longmans, Green and Co., New York, 1929. Price $3.75.

The first edition by these authors appeared in 1922. This volume, dealing with the metabolic processes of plants, has been prepared, as the authors state, "to give such an account as would form a basis for further study." In order not to confuse the student of this subject selected material is presented and the authors "do not profess to have mentioned all research on the subject." They have attempted to give all work of outstanding importance and essential to their presentation of the subjects. While the authors find "the expanding margin of botanical knowledge and the trend of botanical thought have made necessary much revision and rewriting" in this second edition the same treatment of the subject matter as was attempted in the first edition is followed here.

MELLON INSTITUTE ANNOUNCES INDUSTRIAL FELLOWSHIP ON SUGAR

Dr. Edward R. Weidlein, Director, Mellon Institute of Industrial Research, has announced that the institution has lately begun a broad investigation into possible industrial uses for raw and refined sugar. The research will be carried on by a Multiple Industrial Fellowship that will be sustained by The Sugar Institute, Inc., of New York, an organization that represents the cane sugar refiners of the United States.

The comprehensive program of investigation will be supervised by Dr. George D. Beal, Assistant Director of Mellon Institute, and by Dr. Gerald J. Cox, Senior Industrial Fellow. They and the scientists who will be under their direction in

endeavoring to find and to develop uses for sugar in various industries will have the close advisory collaboration of Dr. Leonard H. Cretcher, the sugar specialist who is the head of Mellon Institute's Department of Research in Pure Chemistry.

According to Dr. Weidlein, various studies made by private research workers have already indicated results of industrial promise; these findings will be carefully studied in the laboratories of Mellon Institute. Most of these proposals relate to applications for sugar in such technologic practises as wood preservation, textile finishing, and the manufacture of adhesives. Sugar is thought to merit searching investigation as a basic raw material for employment in various branches of chemical industry.

Four chemists, headed by Dr. Cox, have begun the initial scientific research of the Industrial Fellowship. Additions will be made to this staff, as needed, from time to time.

FIRST DAY

MONDAY—MORNING SESSION

No report on waters, brine and salt was given by the referee. It was recommended that certain changes[1] be made in the official methods and that the study of the quantitative determination of minute quantities of boric acid in waters be continued.

No report on tanning materials and leathers was given by the referee.

REPORT ON INSECTICIDES AND FUNGICIDES

By J. J. T. GRAHAM (Insecticide Control, Food and Drug Administration, Washington, D. C.), *Referee.*

During 1930 the Referee on Insecticides and Fungicides continued the study of methods for the determination of mercury in organic mercurial seed disinfectants. The three methods on which work was reported last year were again studied collaboratively. In methods II and III the digestion apparatus was slightly changed. An Erlenmeyer flask closed with a small funnel was used instead of the ground-in air condenser specified in the 1929 directions. With this exception the methods sent to the collaborators were the same as those reported last year.[2]

Three samples of mercury seed disinfectants obtained from commercial sources were used for the collaborative work. Samples 1 and 2 contained a large quantity of an insoluble filler and in this respect differed from Sample 3, which was entirely soluble.

The collaborative results are given in Table 1. All the collaborators are connected with the U. S. Food and Drug Administration.

The results obtained by Method I are good; excluding the results of one analyst, those reported on Sample 1 show a maximum variation of only 0.31 per cent, and the maximum variation of all the results reported on Sample 2 is only 0.17 per cent. The variation in the results on Sample 3 is somewhat greater, although those reported by six of the eight analysts agree quite closely. There is a wider variation in the results reported by Method II than in those by Method I, although when taken collectively they are satisfactory.

The results obtained by Method III are somewhat erratic, although in a number of instances the analysts obtained results which checked those

[1] *This Journal*, 14, 41, 74 (1931).
[2] *Ibid.*, 13, 156 (1930).

TABLE 1.

Collaborative results—mercury in organic mercurial seed disinfectants.

(Expressed as percentages of metallic mercury.)

ANALYST	SAMPLE 1			SAMPLE 2			SAMPLE 3		
	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
J. C. Bubb	6.00	5.95	5.50	2.41	2.45	2.36	16.60	16.98	19.06
New York	6.00	6.07	5.38	2.43	2.39	2.35	16.60	17.06	18.20
	—	6.09	—	—	—	2.20	16.94	16.92	—
	—	5.95	—	—	—	—	16.40	17.03	—
Average	6.00	6.02	5.44	2.42	2.42	2.30	16.64	17.00	18.63
C. F. Cressy	6.00	5.72	6.96	—	—	—	16.82	16.89	19.23
New York	6.00	5.71	5.99	—	—	—	16.69	16.84	19.93
	—	—	8.57	—	—	—	—	—	20.09
	—	—	7.62	—	—	—	—	—	23.54
Average	6.00	5.72	7.29	—	—	—	16.76	16.87	20.70
Richard Edge	5.35	5.52	5.44	2.35	2.44	3.64	17.19	17.16	16.73
San Francisco	5.45	5.47	5.55	2.26	2.53	3.78	17.04	17.32	16.84
Average	5.40	5.50	5.50	2.31	2.49	3.71	17.12	17.24	16.79
E. C. Haas	—	—	—	2.31	2.29	2.89	17.24	16.58	17.59
New York	—	—	—	2.33	2.28	2.74	17.00	16.67	18.14
	—	—	—	—	2.44	2.89	—	16.76	18.79
Average	—	—	—	2.32	2.34	2.84	17.12	16.67	18.17
F. L. Hart	5.83	5.84	5.79	2.29	2.33	3.30	17.19	17.16	17.40
St. Louis	5.79	5.89	5.69	2.27	2.33	1.65	17.25	17.14	16.50
	5.80	5.86	—	2.32	2.30	—	17.09	17.20	—
Average	5.81	5.87	5.74	2.29	2.32	2.48	17.18	17.17	16.95
J. P. Henry	6.00	6.04	6.27	2.40	2.67	3.07	17.13	17.29	17.59
Washington, D.C.	6.00	5.80	6.69	2.35	2.54	2.79	17.13	17.17	17.70
Average	6.00	5.92	6.48	2.38	2.61	2.93	17.13	17.23	17.65
N. L. Knight	5.80	5.35	6.02	2.35	2.20	3.11	—	17.07	19.05
St. Louis	5.85	3.58*	5.92	2.30	2.33	2.71	17.50	17.24	25.08
	—	—	—	—	—	—	—	—	21.67
	—	—	—	—	—	—	—	—	19.86
Average	5.83	5.35	5.97	2.33	2.27	2.91	17.50	17.16	21.42

* Not included in average.

TABLE 1—(*continued*)

ANALYST	SAMPLE 1			SAMPLE 2			SAMPLE 3		
	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3	Method 1	Method 2	Method 3
C. W. Sondern	—	5.73	5.65	—	2.28	2.40	—	16.98	17.07
Washington,	—	5.57	5.50	—	2.28	2.25	—	16.94	17.22
D.C.	—	5.84	—	—	2.43	—	—	17.09	—
Average	—	5.71	5.58	—	2.33	2.33	—	17.00	17.15
R. D. Stanley	6.10	5.75	7.95	2.28	2.39	—	17.28	17.23	18.75
St. Louis	6.01	5.83	7.19	2.36	2.29	—	16.68	17.16	17.77
	—	—	6.81	—	—	—	17.12	—	—
	—	—	7.27	—	—	—	—	—	—
Average	6.06	5.79	7.31	2.32	2.34	—	17.03	17.20	18.26
General average	5.87	5.79	6.39	2 33	2.38	2.76	16.99	17.04	18.86

obtained by Method I. The titration in Method III should be carried out in a solution having alkaline or neutral reaction. Analysts Hart and Stanley state that in some cases the solutions after boiling showed an acid reaction and they suggest that this is probably the cause of the variation in the results. They both think that the method is sound in principle, and that with further study and more detailed directions it should prove to be satisfactory.

The oxidation procedure followed in Methods II and III proved satisfactory in the case of the samples sent to the collaborators, but when subsequently used on another sample the oxidation was incomplete. It was then necessary to use a flask with a ground-in condenser and to heat it over a low flame as directed in the work last year. In view of this fact it will be better to use an apparatus of this type in future work.

COMMENTS BY ANALYSTS

J. C. Bubb (reporting also for C. F. Cressy and E. C. Haas).—It will be noted that sample 3 gave erratic results by all three methods. Method I appears to give most satisfactory results and is the easiest to handle. In Method II, I experienced some difficulty in washing out the sulfur from the sulfide, and best results were obtained by using smaller samples than specified, and also by washing the sulfides with alcohol and ether. Method III appears to give considerable difficulty and in most cases high results. My results on samples 1 and 2 were determined on smaller samples than specified and by titrating the excess cyanide without the use of an indicator. Sample 3 again showed high and inconsistent results as determined by three analysts.

The weights of sample and detail of analysis were carried out by Cressy and Haas strictly as specified in the methods, with the exception that sample 3 was in most cases weighed by difference from a weighing bottle because of its hygroscopic nature.

Richard Edge.—Perhaps a criticism on the procedure for freeing the mercury should be offered. I believe that more satisfactory results would be obtained if after the digestion the flasks containing the samples and perhydrol-sulfuric acid mixture were heated to boiling for a few minutes. The loss of mercuric chloride is doubtful when present in the concentration that is usual with such samples.

In filtering, the solution should first be run through an open filter, and then refiltered through a tighter filter. In this way, I believe, time can be saved and a clearer solution obtained.

So far as could be determined there is very little, if any, time saved by Method III, and unless a large number of samples are being run, trouble will be experienced in selecting a true end point, particularly if the solutions are somewhat colored.

No criticism of Methods I and II is offered. Of the three methods, I believe Method I to be the best, both as to accuracy and rapidity of analysis.

F. L. Hart.—Methods I and II are admirable. It seems to me that Method III, while apparently sound in principle, lacks sufficient detail. When the directions were followed blindly, erratic results were obtained. I think the alkalinity of the solution just before titration should be controlled. When the samples were run exactly as outlined, my solutions on samples 2 and 3 were acid, and on sample 1 the solution was neutral. According to Scott the solutions should be neutral or alkaline. I therefore added ammonia to alkalinity to litmus. The method says to boil 3 minutes before filtering and titrating. Using ammonia this, of course, will give varying degrees of alkalinity. Would not a measured excess of sodium hydroxide be better?

N. L. Knight.—In Method I, while the second determination was being made, it was noted that a fragment of the mercury film detached itself while the gold crucible was being washed out with ethylalcohol. The crucible was carefully rotated until the fragment adhered again. The low result on the first determination may have been due to an unobserved loss of mercury occurring in the same manner.

In Method II a 2 gram sample of both numbers 1 and 2 was taken in making the first determinations; this was reduced to 1 gram in making the duplicate determinations reported, and a lesser quantity of superoxol was used. The use of an insufficient quantity of superoxol in the second determination on sample 1 may have caused the low percentage obtained.

Method I is the most rapid of the three methods and gives concordant results. Method III stands second as regards rapidity, but the results are very erratic. Method II is the longest of the three, but gives good checks, with an apparent tendency to run somewhat below the values obtained by Method I.

R. D. Stanley.—Method III gave results that were high in comparison with those obtained by Methods I and II. When ready for titration the solutions were frequently noticed to be acid to litmus paper. As Scott says the Liebig titration for cyanides should be made in neutral or alkaline solution, the effect of making the solution alkaline with ammonia and with potassium hydroxide was tried.

After adding 25 cc. of the potassium cyanide solution to the sample and mixing well, 5 cc. of 10 per cent potassium hydroxide solution was added and the solution was titrated with silver nitrate. The same quantity of potassium hydroxide was added to the blank. This procedure gave results of 7.74 per cent for sample 1 and 17.54 and 17.79 per cent for sample 3.

At the end point obtained as directed in Method III, 5 cc. of strong ammonia was added, and the solution was titrated to a new end point. A new blank was obtained by adding 5 cc. of strong ammonia to the same quantity of potassium cyanide solution as was used in the titration of the sample. The results obtained more nearly agreed with those from Methods I and II. However, the concentration of ammonia in the solution seems to affect the titration materially.

Methods I and II seem to be quite satisfactory, but Method III gave high values and the results were quite erratic. Method III is a very convenient method and I think it should be investigated further.

TEXT OF THE METHODS, AS RECOMMENDED ON THE BASIS OF THE 1930 COLLABORATIVE WORK

I.—Volatilization Method[1]

No change recommended.

II.—Precipitation Method[2]

REAGENTS

(a) *Hydrogen peroxide.*—A 30 per cent solution, commonly designated as "perhydrol" or "superoxol."

(b) *Potassium permanganate solution.*—An approximately 0.1 *N* solution.

APPARATUS

Digestion flask.—A 200 cc. Erlenmeyer flask, fitted with an air condenser by means of a ground-glass joint.

DETERMINATION

Place 0.5–2.0 grams of the sample, depending on the quantity of mercury present, in the digestion flask, add 10 cc. of the concentrated sulfuric acid, connect the flask to the condenser, and rotate in order to bring all the sample into contact with the acid. Then add dropwise through the condenser tube 3–5 cc. of the 30 per cent hydrogen peroxide solution, and mix by rotation of the flask. After the active reaction has subsided, heat over a low flame for 15–20 minutes, add 5 cc. more of the hydrogen peroxide and continue the heating until all organic matter is destroyed (indicated by a clear solution), adding more hydrogen peroxide if necessary. Remove the flask from the heat, allow to cool, wash down the condenser, and transfer the contents to a beaker, filtering if necessary. Dilute to about 200 cc. and destroy the excess of hydrogen peroxide by titration with potassium permanganate. Precipitate the mercury with hydrogen sulfide, filter through a weighed Gooch crucible and dry the precipitate in an oven at 105°–110°C. Extract the dried precipitate with carbon disulfide to remove any precipitated sulfur, again dry, and weigh. From the weight of mercury sulfide, calculate the percentage as metallic mercury, using the factor 0.86219.

III.—Titration Method[3]

This method should receive further study, especial attention being paid to the reaction after the boiling with ammonia.

SUGGESTIONS FOR FUTURE WORK

It has been suggested that a study be made of a method for the determination of copper and lead in mixtures of lead arsenate with Bordeaux mixture. The method is based on the solubility of copper compounds and the insolubility of lead arsenate in acetic acid. It is claimed that the method has given satisfactory results and is much more rapid than the present official method.

[1] *This Journal*, 13, 156 (1930).
[2] *Ibid.*, 157.
[3] *This Journal*, 13, 158 (1930).

RECOMMENDATIONS[1]

It is recommended—

(1) That Method I be adopted as an official method for the determination of mercury in organic mercurial seed disinfectants (first action).

(2) That Method II, as described in this report, be adopted as an official method for the determination of mercury in organic mercurial seed disinfectants (first action).

(3) That Method III for the determination of mercury in organic mercurial seed disinfectants be studied further, especial attention being paid to the reaction of the solution at the time of titration.

REPORT ON FLUORINE COMPOUNDS

By G. A. SHUEY (University of Tennessee, Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

Six compounds of fluorine, namely, sodium and potassium fluorides; sodium, potassium and barium fluosilicates; and sodium aluminum fluoride (cryolite), have been successfully used as insecticides.[2] Other compounds of fluorine, including organic fluorides, undoubtedly will be added to the list.

In accordance with the recommendation approved last year[3] the associate referee continued a study of the volatilization method for the determination of fluorine.

PREVIOUS METHODS

The present method was evolved by Wöhler[4] who was the first to estimate fluorine by the formation of silicon tetrafluoride in the presence of silica. He estimated the fluorine from the loss in weight of his generating flask. Other investigators[5] have treated the silicon tetrafluoride in various ways as a measure of the fluorine. Offermann[6] passed the silicon tetrafluoride gas into water and titrated the resulting hydrofluosilicic acid with standard alkali, using phenolphthalein as indicator.

Adolph[7] assembled an apparatus according to the best recommendations prior to this date. His experiments were confined to a study of the conditions that affect the evolution of silicon tetrafluoride. The method of Wagner and Ross[8] presented by title[9] before this association in 1917, embraced the Wöhler-Offermann principle with their modifications. The

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 41 (1931).
[2] Marcovitch, Tenn. Agr. Exp. Sta. Bull., 139 (1928); Marcovitch and Stanley, *ibid.*, 140 (1929).
[3] *This Journal*, 13, 56, 159 (1930).
[4] *Pogg, Ann.*, 48, 87 (1839).
[5] *Z.* Anal. Chem., 5, 190 (1866); 24, 328 (1885); 26, 733 (1887); *Bull. Soc. Chim.*, (2) 50, 167 (1888); *Compt. rend.*, 114, 750, 1189 (1892); *Am. Chem. J.* 1, 27 (1879).
[6] *Z. angew. Chem.*, 3, 615 (1890).
[7] *J. Am. Chem. Soc.*, 37, 2504 (1915).
[8] *J. Ind. Eng. Chem.*, 9, 1116 (1917).
[9] *This Journal*, 4, 98 (1920).

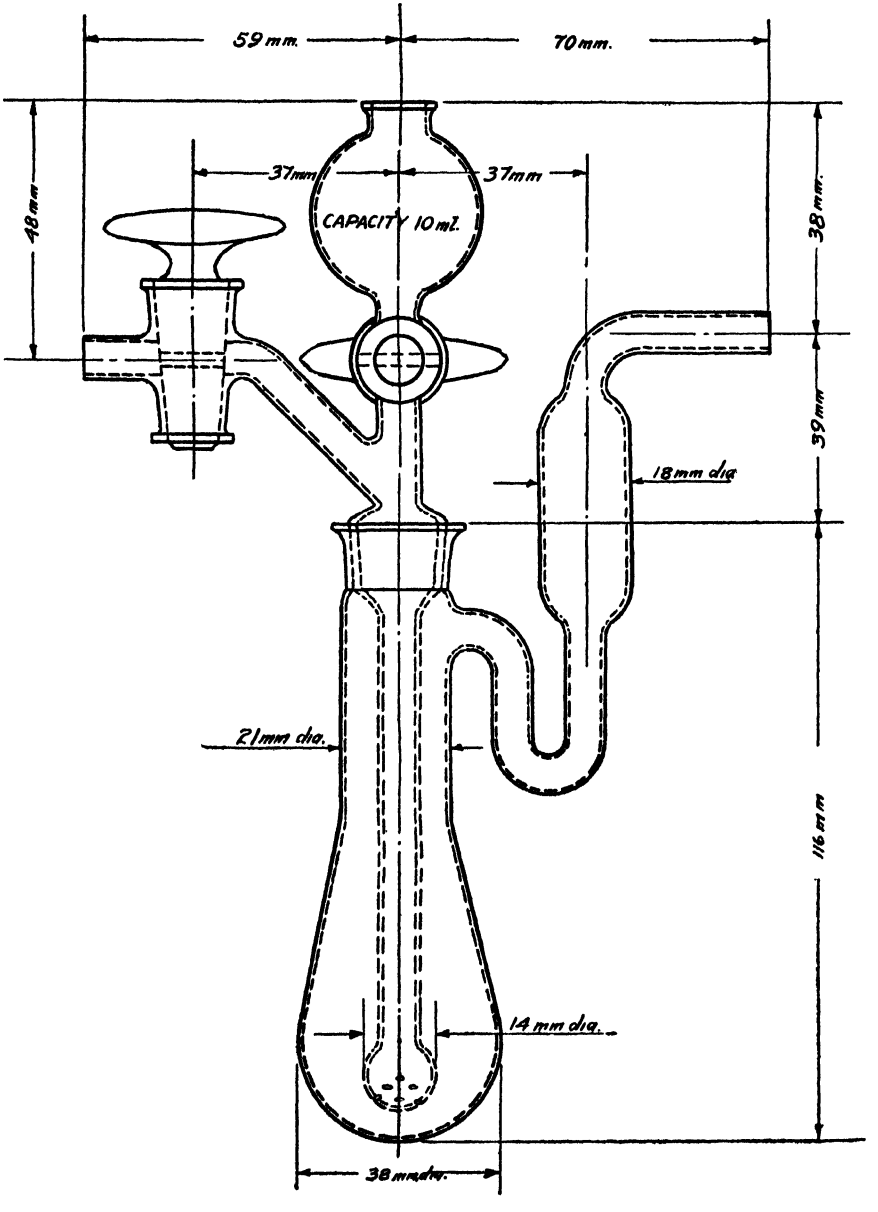


FIG. 1.—REACTION FLASK FOR THE DETERMINATION OF FLUORINE.

Wagner-Ross method, as modified by Patten[1] and Morton,[2] is the present tentative method for the determination of fluorine in baking powder and baking powder ingredients.[3] The most recent modification of the volatilization method is the improved reaction tube and furnace for heating, as recommended by Reynolds, Ross and Jacob.[4] These authors report a recovery of 92–94 per cent of the fluorine present in pure calcium fluoride.

In previous reports[5,6] the associate referee expressed the belief that the volatilization method was superior to other methods in point of speed, ease of manipulation, and applicability to the analysis of numerous fluorine compounds. This method is based on the principle that fluorine is volatilized as silicon tetrafluoride when the compound is mixed with silica and heated with concentrated sulfuric acid. The silicon tetrafluoride thus formed is received in water, and the resulting hydrofluosilicic acid is titrated with standard alkali, phenolphthalein being used as indicator.

PRESENT STUDIES

Of the factors that affect the accuracy of the method, the exclusion of moisture, in so far as possible, from the field of reaction is probably of greatest importance. It cannot be overlooked, however, that water is one of the products of the reaction. It is, therefore, essential that this vitiating factor be reduced to a minimum. With this as an objective the associate referee has designed a reaction flask (Fig. 1) of small size, which permits the introduction of sulfuric acid without removal of the stopper and thus excludes atmospheric moisture. This flask was used as the evolution chamber in the train illustrated in Fig. 2.

REAGENTS[7]

(a) *Sulfuric acid, 98–98.5 per cent.*—Prepare by one of the following methods:

(1) Add sufficient fuming sulfuric acid to ordinary concentrated sulfuric acid to give a solution containing about 99 per cent, as determined by titration. Heat in an open casserole for 1 hour after the appearance of dense fumes. Adjust the acid to contain 98–98.5 per cent by the addition of either ordinary or fuming sulfuric acid, as determined by titration. Protect the acid from atmospheric moisture.

(2) Boil ordinary (approximately 95 per cent) sulfuric acid in an open casserole to about two-thirds its orginal volume. Determine the strength by titration. Protect the acid from atmospheric moisture.

(3) Heat ordinary (approximately 95 per cent) sulfuric acid in a retort until about one-tenth of the original volume has boiled off. Determine its strength and protect from the atmosphere as usual.

[1] *This Journal*, 4, 538 (1921).
[2] *Ibid.*, 8, 101 (1924).
[3] *Methods of Analysis, A.O.A.C.*, 1925, 312.
[4] *This Journal*, 11, 225 (1928).
[5] *Ibid.*, 12, 141 (1929).
[6] *Ibid.*, 11, 147 (1928).
[7] Hydrochloric acid and oxides of nitrogen and sulfur are absorbed, the former in silver sulfate and the latter in chromic-sulfuric acid. Asbestos contained in tube (I) serves to condense traces of sulfur trioxide that may escape absorption in the chromic-sulfuric acid solution.

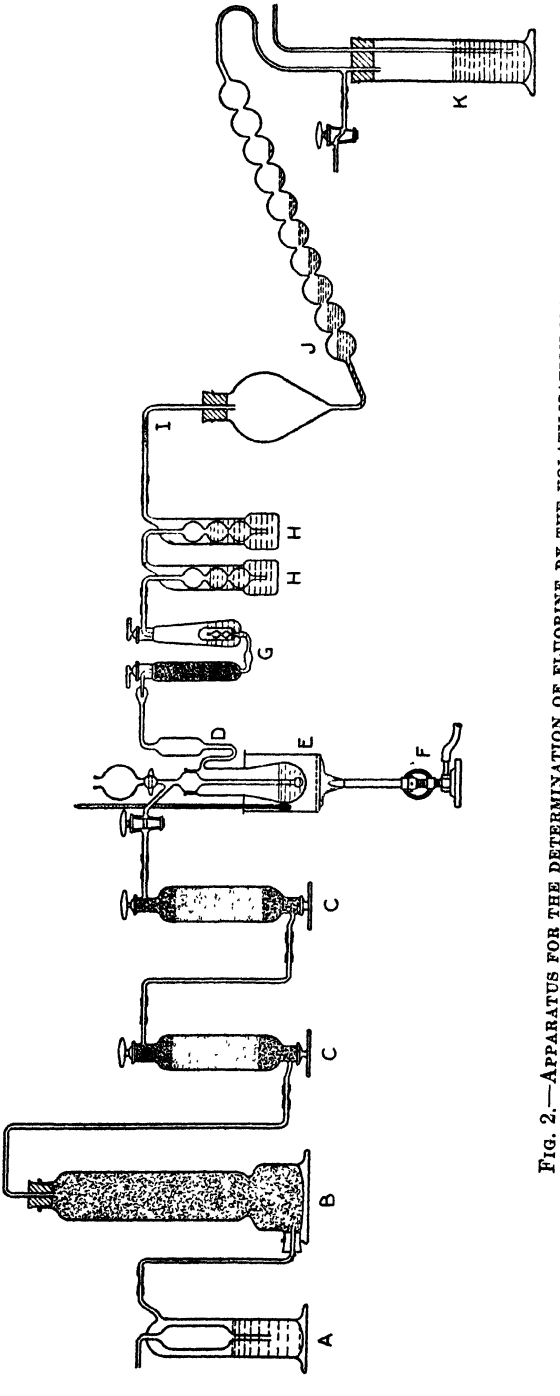


FIG. 2.—APPARATUS FOR THE DETERMINATION OF FLUORINE BY THE VOLATILIZATION METHOD.

(b) *Quartz flour (silica).*—Grind ordinary quartz to 200-mesh and ignite at a red heat.

(c) *Silver sulfate in sulfuric acid.*—Dissolve 10 grams of pure, dry silver sulfate in 100 cc. of sulfuric acid prepared as in (a).

(d) *Chromium trioxide in sulfuric acid.*—Add finely powdered chromium trioxide, previously dried at 110°C., to 100 cc. of sulfuric acid prepared as in (a), permitting an excess of the powder to remain in suspension.

(e) *Asbestos.*—Ignite 5 grams of acid-washed asbestos of medium fiber to a red heat.

(f) *Sodium hydroxide solution.*—0.1 *N*.

APPARATUS

As assembled it consists of the following: (A), wash bottle containing concentrated sulfuric acid, for initial drying of the ingoing air; (B), calcium chloride jar filled with 4-mesh, granular calcium chloride; (C) (C), jars loosely filled with phosphoric anhydride, with tops and bottoms protected by layers of ignited asbestos; (D), reaction flask detailed in Fig. 1; (E), metal air bath, with asbestos board cover to protect the upper part of the flask; (F), gas burner (a hot plate or furnace may be substituted); (G), Schmitz tube (4 inch) containing glass beads in one arm, and 5 cc. of silver sulfate solution in sulfuric acid in the other arm; (H) (H), Bowen bulbs containing 25 cc. of chromium trioxide in sulfuric acid; (I), glass tube (10 × ⅝ inches) containing ignited asbestos, as illustrated; (J), Meyer absorption bulb containing 50 cc. of water for collection of the silicon tetrafluoride; (K), pressure regulator containing mercury. (It is imperative that joints be made airtight by bringing all apparatus tubes in close contact within rubber tubing connectors.)

DETERMINATION

Preliminary treatment of sample.

(a) If organic matter is present, weigh into a platinum dish a quantity of the finely ground material equivalent to about 30–40 mg. of fluorine. Add about 0.5 gram of pulverized calcium oxide and sufficient water to form a smooth paste, mix thoroughly, dry, and ignite in a muffle furnace at 500°C. until a white ash is obtained. Protect in a desiccator.

(b) For substances that contain no organic matter proceed without preliminary treatment other than pulverizing and drying. (The method is not suited for the determination of fluorine in substances containing boron.)

Place the sample prepared under (a) or (b), together with 1 gram of 200-mesh quartz flour, in an agate mortar, and mix thoroughly. Transfer, through a powder funnel, to the dry reaction flask (*D*), (Fig. 2) and place the flask in its position in the train. Add 50 cc. of water to the Meyer absorption bulb (*J*), and connect as illustrated. Apply suction and permit a current of air to pass through the apparatus at the rate of three bubbles per second, for a period of 30 minutes. Maintain this rate of aeration throughout the entire determination. Add through the funnel of the reaction flask about 10 cc. of anhydrous sulfuric acid, reagent (a), retaining in the funnel a few drops of the acid. (If the ash content of the sample is high, add the acid very slowly.) Allow the mixture to stand 10 minutes and shake at least twice during this interval. Apply heat to the reaction flask, slowly bringing the temperature to about 220°C. (as indicated by a thermometer supported in a position such that its mercury bulb is in contact with the flask at a point below the surface of the acid), and maintain this temperature for 1 hour. Shake the flask every 5 minutes until the gray-white scum, which forms on the surface, completely disappears. After 1 hour increase the temperature and boil for a period of 5 minutes. Remove the heat but

continue to aerate for 30 minutes, to recover any silicon tetrafluoride remaining in the system. Disconnect the absorption bulb (a short auxiliary tube of glass with stopcock connecting (I) and (J) is advantageous in preventing back pressure while (J) is being disconnected) and transfer its contents with rinsings to a 500 cc. Erlenmeyer flask. Dilute with water to about 250 cc., heat to boiling, and boil gently for 5 minutes. Titrate the hot solution with 0.1 *N* sodium hydroxide, using phenolphthalein as indicator. 1 cc. of 0.1 *N* sodium hydroxide = 0.0019 gram of fluorine. Conduct a *blank determination* and correct for the volume of 0.1 *N* alkali required in the blank.

To study the accuracy of the method with the modifications as described, a sample of Baker's C.P. analyzed sodium fluoride was used. The salt was thoroughly dried and pulverized to pass a 100-mesh sieve. The data given in the following table indicate the further possibilities of this

Recovery of fluorine from different quantities of sodium fluoride.

SAMPLE TAKEN		FLUORINE RECOVERED	
Sodium fluoride	Fluorine		
gram	*gram*	*gram*	*per cent*
0.0174	0.00787	0.00770	97.84
0.0195	0.00882	0.00879	99 65
0.0224	0.01013	0.01017	100 39
0.0439	0.01985	0.01932	97.33
0.0555	0.02511	0.02379	94.74
0.0562	0.02542	0.02501	98.38
0.0615	0.02782	0.02605	93.27
0.0623	0.02818	0.02660	93.63
0.0665	0.03008	0.02800	93 08
0.0697	0.03153	0.03022	95.81
0.0801	0.03624	0.03397	93.73
0.0835	0.03777	0.03777	100.00
0.1058	0.04786	0.04600	96.11
0.1286	0.05817	0.05222	89.77
0.1316	0.05953	0.05483	92.10
		Av.	95.72

method. The better and more consistent recoveries seem to have been obtained when quantities of sodium fluoride equivalent to 17–43 mg. of fluorine were used. In the fifteen determinations conducted, after correcting for blanks, an average fluorine recovery of 95.72 per cent was obtained. The average result would suggest the use of 1.043 as a factor in placing the recovery of fluorine on a 100 per cent basis.

The method described appears to lend itself to the analysis of fluorine compounds in general. Further study of conditions with respect to secur-

ing more intimate contact of interacting materials and minimizing the effect of the water that results as a by-product of the reaction should be made.

RECOMMENDATIONS[1]

It is recommended—

(1) That the modified method for the determination of fluorine in insecticides presented in this report be adopted as a tentative method.

(2) That collaborative and experimental study of this method be conducted next year.

REPORT ON CAUSTIC POISONS

By J. J. T. GRAHAM (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The work on caustic poisons for 1930 was a collaborative study of the Chapin method for the determination of phenol. This is a very important determination from the standpoint of the Caustic Poison Act, which sets a limit of 5 per cent on the phenol content of any preparation sold in packages suitable for household use.

Three samples were prepared for collaborative work; their composition is shown in Table 1.

TABLE 1.

Composition of collaborative samples.

INGREDIENTS	SAMPLE 1	SAMPLE 2	SAMPLE 3
	grams	*grams*	*grams*
Phenol	20	28	40
Cresol	145	48	40
Soap	165	100	
Water	70		
Coal tar neutral oil		224	
Kerosene			280
Oil of birch			40

The cresol in these samples was prepared by partially extracting a commercial cresol several times with a solution of sodium hydroxide and then fractionally distilling the residue, as described in the referee's report for 1928[2]. In this way a sample was obtained which showed only 0.5 per cent phenol when analyzed by the Chapin method. The phenol used was redistilled and had a congealing point of 41° C. The coal tar neutral oil was extracted with a solution of sodium hydroxide, and when analyzed it showed a phenol content of 0.1 per cent.

The three samples were sent to collaborators with the following directions for analysis:

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 41 (1931).
[2] *This Journal*, 12, 143 (1929).

PHENOL

METHOD I[1]

(Applicable to the determination of phenol in commercial cresols, saponified cresol solutions, coal tar dips and disinfectants and kerosene solutions of phenols, except in the presence of salicylates or beta-naphthol.)

REAGENTS

(a) *Dilute nitric acid.*—Blow air through strong nitric acid until it is colorless, then dilute one volume of this acid with four volumes of water.

(b) *Millon's reagent.*—Treat 2 cc. of mercury in a 200 cc. Erlenmeyer flask with 20 cc. of strong nitric acid. Place the flask under a hood and after the first violent reaction is over, shake as much as necessary to effect subdivision of the mercury and maintain action. After about 10 minutes, when the action has practically ceased, even in the presence of undissolved mercury, add 35 cc. of water. If basic salt separates, add sufficient dilute nitric acid to dissolve it. Next add a 10 per cent solution of sodium hydroxide dropwise with thorough mixing until the curdy precipitate following a single drop no longer redissolves but disperses to an evidently permanent turbidity. Then add 5 cc. of dilute nitric acid and mix well. The solution deteriorates and should not be used later than the day following the day of preparation.

(c) *Standard phenol.*—Prepare a stock solution by dissolving a weighed quantity of the pure substance possessing a congealing point of not lower than 40°C. in sufficient water to make not less than a 1 per cent solution. From this stock solution make an 0.025 per cent solution in additional distilled water. This second solution constitutes the final standard, and it should be prepared on the day of use.

(d) *Dilute formaldehyde solution.*—Dilute 2 cc. of commercial 37 per cent formaldehyde solution to 100 cc. with distilled water.

APPARATUS

(a) *Nessler cylinders.*—50 cc. tall-form, matched.

(b) *Test tubes.*—About 180 mm. × 20 mm., marked at 25 cc. and provided with rubber stoppers.

(c) *Water bath.* For heating the test tubes. This may be extemporized from a beaker containing a disk of wire gauze raised somewhat from the bottom.

PREPARATION OF THE SAMPLE FOR ANALYSIS

Commercial Cresol.—Weigh by difference about 2.5 grams of sample into a 250 cc. volumetric flask, dissolve in 10 cc. of a 10 per cent sodium hydroxide solution, and make to the mark with water.

Saponified Cresol Solutions, Coal Tar Dips and Disinfectants, Kerosene Solutions of Phenols, Etc.—Weigh by difference about 5 grams (or use 5 cc. and calculate the weight from the density of the sample) of sample into a 250 cc. volumetric flask and dilute to the mark with water. In products consisting largely of kerosene, bring the water level to the mark and take aliquots from the aqueous portion only.

DETERMINATION

Transfer a 5 cc. aliquot of this solution to a 200 cc. volumetric flask shortly before the determination is to be carried out, dilute to about 50 cc., add one drop of methyl orange indicator solution and then dilute nitric acid until the solution is practically neutral, make to volume, and shake well.

[1] Chapin, U. S. Dept. Agr. Bull. 1308, p. 17.

Place 5 cc. of the diluted solution in each of two of the marked test tubes, and in each of two additional test tubes place 5 cc. of the standard phenol solution (c). Next flow 5 cc. of Millon's reagent (b) down the side of each tube, mix, and place the tubes in a bath of boiling water; continue the boiling for exactly 30 minutes, cool immediately and thoroughly by immersion in a bath of cold water for at least 10 minutes, and then add 5 cc. of dilute nitric acid (a) to each tube.

After brief mixing add 3 cc. of dilute formaldehyde solution (d) to one of each pair of tubes, make all the tubes to the 25 cc. mark with water, stopper them, shake each one well, and put them all aside to stand overnight. The next day the contents of the tubes to which formaldehyde was added will have faded to a yellow, while the others will possess an orange or red tint.

Pipet 20 cc. from each of the two phenol tubes and transfer to 100 cc. volumetric flasks, treat each with 5 cc. of the dilute nitric acid, make to the mark, and mix. The red flask contains the "phenol standard," and the yellow flask the "phenol blank." Transfer these solutions to burets. Pipet 10 cc. of each sample solution into Nessler tubes. The orange or red one constitutes the "unknown," and the yellow one the "sample blank," and each Nessler tube must be distinctly marked to avoid confusion. Next add to the sample blank tube, a measured quantity of "phenol standard" from its buret and add the same volume of "phenol blank" to the unknown, thoroughly agitate (aided by insertion of the rubber stoppers if necessary), and compare the colors. When the tubes have in this way been brought to a match, each cc. of the phenol standard employed is equivalent to 1 per cent of phenol if a portion of sample weighing exactly 5 grams was used, or 2 per cent if exactly 2.5 grams was used.

NOTE.—In using this method the following precautions should be taken: A pair of phenol tubes affords sufficient final solutions for assaying several unknowns, but all of the latter must have accompanied the phenol solutions throughout the entire process with identical reagents and treatment. If the end point has been inadvertently overrun it is possible to work back to it; but, since mistakes are easy to make in this procedure, it is better to repeat the comparison on fresh portions from the original tubes. Too much delay in matching the tubes must be avoided once the titration has been started or the excess of formaldehyde remaining in the blanks may have time after mixture to affect the intensity of the red color. Millon's reagent is dangerously poisonous and should not be transferred with an ordinary pipet and mouth suction unless a protective trap of some kind is used.

METHOD II[1]

(Applicable to the determination of phenol in the presence of salicylates.)

REAGENTS

The reagents are described under Method I.

DETERMINATION

Weigh by difference about 10 grams (or use 10 cc. and calculate the weight from the density of the sample) of sample into a separatory funnel, add 50 cc. of kerosene, and extract three times with 100 cc. portions of water. Filter the aqueous extracts through a wet filter into a 500 cc. volumetric flask, make to volume with distilled water, and proceed as directed in Method I, beginning with "Transfer a 5 cc. aliquot of this solution to a 200 cc. volumetric flask."

[1] Hamilton and Smith, *Ind. Eng. Chem., Anal. Ed.*, 1, 232 (1929).

When the tubes have been brought to a match, each cc. of the phenol standard employed is equivalent to 1 per cent of phenol if a portion of the sample weighing exactly 10 grams was used.

The precautions mentioned in Method I should also be observed in carrying out this method.

The collaborative results are given in Table 2.

TABLE 2.

Collaborative results—phenol in caustic poisons.

ANALYST	METHOD I		METHOD II
	Sample 1	Sample 2	Sample 3
	per cent	*per cent*	*per cent*
C. F. Cressy	5.1	7.3	10.1
New York	5.0	7.4	10 2
Average	5.1	7.4	10 2
C. G. Donovan Washington, D.C.	5.5	7.0	10.2
Richard Edge San Francisco	5.1	7.1	9.9
J. J. T. Graham	5.8	7.5	10.0
E. C. Haas	4.8	7.4	10.4
New York	4.7	7.1	9.1
	4.9		
	4.9		
Average	4.8	7.3	9.8
J. P. Henry	5.4	7.8	10.2
Washington, D.C.		7.9	10.4
Average	5.4	7.9	10.3
N. L. Knight	5.5	7.8	12.3*
St. Louis	5.5	7.2	12.0*
Average	5.5	7.5	—
W. J. Morgan Washington, D.C.	5.4	7.8	10.0
R. D. Stanley	5.0	7.8	11.6*
			11.0
St. Louis	5.2	7.7	10.8
			10.9
Average	5.1	7.8	—
General Average	5.2	7.5	10.2
Calculated percentage	5.2	7.1	10.1

* Not included in average.

DISCUSSION

The method recommended by the previous referee was a combination of Methods I and II as used in this report. His object was to formulate a method that would serve for all classes of samples. However, it has seemed to the present referee that it would be better to separate that method again into the two original methods. When this is done, the longer procedure of Method II will only be necessary in the case of those samples in which methyl salicylate occurs. This class of samples constitutes only a minor proportion of caustic poison samples and therefore much time will be saved.

In dividing the method there has been no change in principle, and any minor changes are only of an editorial nature. Nine analysts from the U. S. Food and Drug Administration assisted in the work, and the results in the table show that the methods are correct to about 1 part in 10, which is fairly satisfactory for a colorimetric procedure. There is a tendency for these methods to run high if too much time is consumed in matching the tubes. In such cases the formaldehyde still present in the blanks will exert a bleaching effect, necessitating the addition of an excess of the standard in order to get a perfect match of the tubes. Several cases in which the analyst reported results somewhat above the 10 per cent limit were probably due to this cause.

SUGGESTIONS FOR FUTURE WORK

It is suggested that future work be directed along the lines suggested by the previous referee—the development of methods for the estimation of "free or chemically unneutralized" acids and "free and chemically uncombined" ammonia.

RECOMMENDATIONS[1]

It is recommended—

(1) That Method I be adopted as an official method for the determination of phenol (carbolic acid) in such products as cresol, saponified cresol solutions, coal tar dips, disinfectants, etc. (final action).

(2) That Method II be adopted as an official method for the determination of phenol (carbolic acid) in the presence of methyl salicylate in such products as fly sprays, disinfectants, etc. (final action).

REPORT ON SOILS AND LIMING MATERIALS

By W. H. MacIntire (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Referee*

The work during the past year has been a study of possible refinements in the present methods, deletion of unused, or unnecessary, procedures

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 41 (1931).

and the development of new methods. The work of two Associate Referees, W. M. Shaw and J. S. McHargue, has been responsible for many improvements and the development of new methods.

To bring the status of the two chapters, Soils and Liming Materials, to date and with the concurrence of the Committee on Revision of Methods of Soil Analysis, it is recommended—

(1) That sec. **5**, p. 21, Loss on Ignition, method 2 be deleted.

(2) That par. (**b**) and Fig. 4, p. 23, Carbonate Carbon, be deleted.

(3) That sec. **8**, p. 24, Carbonate Carbon, be modified: to specify 60-mesh in lieu of 100-mesh; to specify 60 minutes agitation and aspiration in lieu of 30 minutes; to stipulate that the 1+9 HCl shall contain 5 per cent of stannous chloride; to stipulate the rate of aspiration and to delete the two sentences beginning "If the barium carbonate" and insert "and let stand 4 hours" after the words "Make to volume, agitate."

(4) That Method II, Gravimetric, as sec. **7**, p. 24, Carbonate Carbon, be inserted with the further insertion of a paragraph relative to special consideration to be given soils that have received, or carry, magnesite or dolomite.

(5) That sec. **8**, *Furnace Combustion Method, Organic Carbon*, be inserted.

(6) That sec. **11**, p. 25, Dry combustion method, organic carbon, be deleted.

(7) That sec. **15**, p. 28, be rewritten to provide for the complete precipitation of manganese along with the iron, aluminum, phosphorus and titanium by the use of ammonium persulfate and for editorial changes.[1]

(8) That Method II be inserted after sec. **24**, p. 30, Manganese, as recommended at this meeting.

(9) That a new section be inserted following Manganese, Method II, to provide for the determination of iodine.

LIMING MATERIALS

(10) That minor editorial changes be made as designation of quantities for charges and insurance against evolution of H_2S.[1]

(11) That sec. **6**, Method II, p. 36, be deleted.[1]

(12) That Method I, sec. **5**, p. 36, Calcium oxide in burnt and hydrated lime be adopted as official.[1]

(13) That further work be done in the perfection of methods for the determination of the less abundant elements in soils.[2]

No report on the reaction value of alkaline and acid soils was given by the associate referees.

[1] Previous affirmative action by the association.

[2] For report of Subcommittee A and action of the association, see *This Journal*, **14**, 42 (1931).

REPORT ON LIMING MATERIALS

By W. M. SHAW (University of Tennessee Agricultural Experiment Station, Knoxville, Tenn.), *Associate Referee*

The following experimental work was carried out following the 1929 recommendations of the Referee on Soils and Liming Materials that "provision be made to insure against the error introduced by hydrogen sulfide in the determination of carbonate carbon dioxide."[1]

Experiments were carried out with additions of pyrite, sphalerite and fused ferrous sulfide to charges of limestone to determine their decomposibility in hydrochloric acid (1+9), both at room and boiling temperatures. Other experiments were performed to determine the efficacy of a silver sulfate suspension in dilute sulfuric acid to insure complete elimination of hydrogen sulfide from the gas current. The results may be summarized as follows: Pyrite was not attacked by the hydrochloric acid either at room temperature or on boiling. Sphalerite was not attacked at room temperature, but was readily decomposed in the hydrochloric acid on boiling. Fused ferrous sulfide was slowly decomposed by hydrochloric acid (1+9) at room temperature and to a much greater extent on boiling. The bubbling air, strongly charged with hydrogen sulfide, through a silver sulfate suspension at the maximum rate permissible for carbon dioxide determinations failed to reveal any hydrogen sulfide in the escaping gases by the ammoniacal cadmium chloride test.

It is recommended[2] that a silver sulfate suspension in dilute sulfuric acid (1+19) be used in the carbon dioxide absorption train to insure removal of any hydrogen sulfide that may be evolved.

REPORT ON LESS COMMON METALS IN SOILS

BY J. S. MCHARGUE[3] (Department of Chemistry, Kentucky Agricultural Experiment Station, Lexington, Ky.), *Associate Referee*

Tentative methods for the determination of manganese, copper and zinc in soils were proposed at the meeting last year, and further collaborative work was anticipated. The assistance of one collaborator was obtained in addition to the work done under the direction of the associate referee. Good results were reported for manganese in soils by all the collaborators. However, the results for the copper and zinc in the sample of soil varied so widely that it is desirable to repeat the work next year.

It is recommended that the following method for the determination of manganese in soils be made permanent:

[1] *This Journal*, 13, 57 (1930)
[2] For report of Subcommittee A and action of the association, see *This Journal*, 14, 42 (1931).
[3] Presented by W. H. MacIntire.

MANGANESE

REAGENTS

(a) *Sulfuric acid solution (1+1).*

(b) *Potassium bisulfate.*—Manganese-free and finely pulverized.

(c) *Standard manganous sulfate solution.*—Dissolve 0.2877 gram of pure potassium permanganate in about 100 cc. of water, acidify the solution with dilute sulfuric acid (1+1), and slowly heat to boiling. Add slowly a sufficient quantity of a 10 per cent oxalic acid solution to discharge the color. Cool, and dilute to 1 liter. 1 cc. of this solution = 0.1 mg. of manganese.

(d) *Potassium periodate.*

DETERMINATION

Weigh 0.5–5.0 grams of finely pulverized, air-dried soil into a 50 cc. silica or platinum crucible. Add to the soil approximately 2½ times its weight of finely powdered, manganese-free potassium bisulfate and mix thoroughly. Place the lid on the crucible and heat gently over a Bunsen burner for about 5 minutes; increase the heat gradually until the crucible and lid are red hot, being careful not to allow the contents of the crucible to froth over. Continue to heat for about 20 minutes, or until the frothing has ceased and the contents are in a quiet molten condition. Withdraw the flame from beneath the crucible, remove the lid, and rotate the crucible in a horizontal position to spread the molten contents over the inner walls to expedite cooling. When the crucible is no longer red, immerse it in about 25 cc. of sulfuric acid (1+1) in a 250 cc. beaker and digest over a hole on a hot water bath until the contents of the crucible disintegrate and dissolve. Carefully rinse the crucible and lid with hot water and dilute the solution to about 100 cc. Filter, and wash the insoluble residue.

Discard the insoluble residue if it has a uniform white color. If it is colored by undecomposed particles of minerals, ignite and expel the silica with hydrofluoric and sulfuric acids. Fuse the residue with potassium bisulfate, digest in dilute sulfuric acid, and add the solution to the filtrate from the fusion.

Make the solution that contains all the manganese to a definite volume and take an aliquot for the determination. Add about 0.05 gram of potassium periodate to the aliquot. Boil the solution until the characteristic purplish permanganic acid color develops, heat on a hot water bath for an hour, and set aside to cool. If the color is deep purple, dilute the solution to a definite volume. Remove an aliquot and match against a standard manganese solution in Nessler jars or in a colorimeter. Compute the results as percentage of manganese (Mn or Mn_3O_4). (A series of manganese standard solutions are prepared from reagent (c) by removing aliquots of the manganous sulfate solution and developing the manganese color with potassium periodate in the same way as for the solution of the sample.)

Experiments were made to ascertain if a digestion of a portion of a soil in strong hydrochloric and nitric acids was as effective in dissolving manganese, copper and zinc as the bisulfate fusion. It was found that the filtrate from the acid digestion contained considerable organic matter which interfered with the copper and zinc precipitation, and that it also required more time for manipulation and did not give as high results as did the bisulfate fusion method.

A METHOD FOR THE DETERMINATION OF IODINE IN LIMESTONE ROCKS, ROCK PHOSPHATE AND SOILS

It occurred to the associate referee that possibly iodine could be distilled from limestone rocks, phosphate rock and soils at a rather high tem-

perature. Accordingly, an electric combustion tube furnace that produced a maximum temperature of about 1100°C. was obtained. Fifty grams of material was ignited for about 4 hours in a quartz combustion tube in the furnace, and the volatile matter was aspirated through a 10 per cent solution of potassium carbonate held in gas wash bottles. After ignition the potassium carbonate solution containing the iodine was evaporated to dryness in a porcelain dish and ignited gently to char the trace of organic matter that is usually present. The residue was cooled, moistened with 95 per cent alcohol, ground fine in a small procelain mortar, transferred to a small filter, and washed with small portions of alcohol until the filtrate contained about 20 cc. The filtrate was evaporated to dryness in a small beaker, 1 cc. of a solution of sulfurous acid was added to the residue and evaporated to dryness, and the residue was dissolved in a few cc. of water and filtered into a small separatory funnel; 1 cc. of carbon disulfide, measured accurately, and approximately 1 cc. each of dilute sulfuric acid and a 10 per cent solution of sodium nitrite was added, and the flask was stoppered tightly and vigorously shaken for about 1 minute. If iodine is present, the carbon disulfide is colored pink. An aliquot of a standard potassium iodide solution was treated in the same way in another separatory funnel, and portions of the sample and standard carbon disulfide solutions were matched in a microcolorimeter. The result was calculated to parts per billion of iodine in the sample.

The following results were obtained:

	Iodine *p.p.b.*
Cultivated bluegrass soil	813
Duplicate	720
Average	767
Cultivated sandy soil	312
Duplicate	297
Average	305
Nearby virgin sandy soil	676
Duplicate	554
Average	615

The same procedure was used for the determination of iodine in limestone rock and rock phosphate, 100 and 25 grams, respectively, being used for the determinations. The following results were obtained.

	p.p.b. Iodine
Calcite, 3 different samples	0
Crystalline limestone	0
Impure gray limestone	147
Magnesian limestone	168
Oolitic limestone	800
Oystershells	892
Tennessee rock phosphate	5,450
Kentucky rock phosphate	6,720
Phosphatic clay (15%B.P.L.)	2,100

It appears from the results given that the electric distillation method is applicable for the determination of iodine in soils, limestones and rock phosphate.

It is recommended[1] that the method be made tentative and given further study.

REPORT ON FEEDING STUFFS

By V. E. MUNSEY (U. S. Food and Drug Adm., Washington, D. C.), *Referee*

The Referee on Feeding Stuffs carefully studied the results of the work and the recommendations of the referees made during the past few years. He finds that some methods were studied and later dropped. Some of the present tentative methods might be made official as a result of the work already done, although some need more study and cooperative testing. Accordingly the recommendations that follow pertain to these methods as well as to the collaborative work carried on during the year by the associate referees. A complete report of the associate referees will be presented in detail, which obviously eliminates repetition at this time. The referee recommends[1]

(1) That the method of preparation of solution and determination of sugars in feeding stuffs be adopted as official (first action).

(2) That the methods for the determination of hydrocyanic acid formed by the hydrolysis of glucoside-bearing material be further studied.

(3) That the method for the determination of dried buttermilk in feeding stuffs by the identification of the lactic acid bacilli be discontinued.

(4) That the 135°C. air oven method for the determination of moisture in feeding stuffs that do not contain sugar, be adopted as official (first action).

(5) That the proposed method for the determination of calcium oxide in mineral feeds be adopted as tentative and that work be continued.

(6) That further work on the determination of iodine in mineral feeds be carried on by the Knapheide and Lamb method and other methods proposed for consideration.

(7) That the method for the determination of iodine in organic materials be studied.

(8) That the study of the methods for the detection of traces of ferrous sulfate, copper sulfate and potassium iodide in feeding stuffs be continued next year.

(9) That a study of the Sterling method for the determination of hoof meal be placed in abeyance for next year pending adoption of modifications for the improvement of the method.

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 42 (1931).

REPORT ON STOCK FEED ADULTERATION

By HOWARD E. GENSLER (Department of Agriculture, Harrisburg, Pa.), *Associate Referee*

The associate referee sent copies of the Sterling method for the quantitative determination of hoof meal in animal by-product feeds and also copies of methods devised by him for the detection of ferrous sulfate, copper sulfate, or potassium iodide in mixed feeds to seventeen analysts who intended to participate in this work.

These analysts also received a sample of hoof meal, No. 1, and a sample of a 3 per cent mixture of hoof meal and ground cracklings, No. 2, to be analyzed according to the Sterling method. Nine submitted the results of their work, as given in Table 1.

TABLE 1.

Quantitative determination of hoof by the Sterling method.

ANALYST	SAMPLE NO. 1—HOOF MEAL		SAMPLE NO. 2—3% HOOF MEAL	
	Number of Tests	Average Results	Number of Tests	Average Results
		per cent		*per cent*
J. W. Bowen St. Louis, Mo.	5	104.60	4	4.82
C. S. McCullough Ottawa, Canada	8	98.88	4	2.90
Hugh J. Hennessy St. Paul, Minn.	3	96.70	4	4.85
A. P. Kerr Baton Rouge, La.	1	64.85	1	2.83
Willis S. Hilpert Chicago, Ill.	1	99.93	1	4.37
W. T. Mattison Clemson College, S. C.	2	99.66	2	9.21
George K. Redding Detroit, Mich.	2	101.36	2	7.93
V. E. Munsey Washington, D. C.	4	107.19	4	3.98
W. F. Walsh Geneva, N. Y.	2	101.34	2	3.73
O. B. Winters E. Lansing, Mich.	3	105.20	3	7.40
H. R. Kraybill Lafayette, Ind.	2	98.98	3	4.51

A study of the figures in Table 1 indicates such a wide variation in the amount of hoof found and that known to be present that reliance cannot be placed on results obtained by different analysts using the Sterling method. It will be recalled that results obtained in last year's study of the method warranted the same conclusion. The associate referee believes

that much experience in the use of the method is necessary before trustworthy results can be obtained.

No samples were prepared for the application of the methods for identification of traces of ferrous sulfate, copper sulfate, and potassium iodide in feeds. The collaborators were instructed to add these salts to feeds and reduce the amount until the limit of detection was reached. The methods follow:

METHODS FOR IDENTIFICATION OF SMALL QUANTITIES OF VARIOUS CHEMICALS IN FEEDING STUFFS

FERROUS SULFATE

Sift a portion of the feed through a fine sieve (40-mesh) over a sheet of white glazed paper whose entire surface has been moistened with a solution of potassium ferricyanide (1:10) in such a manner that the feed will be distributed thinly over the area of the paper. After a few moments wash off the feed under a slow stream of water.

A blue speck or spot denotes a particle of ferrous salt.

COPPER SALTS

Sift a portion of the feed through a fine sieve (40-mesh) over a sheet of white glazed paper whose entire surface has been moistened with a solution of potassium ferrocyanide (1:10) in such a manner that the feed will be distributed thinly over the area of the paper. After a few moments wash off the feed under a slow stream of water.

A brown speck or spot denotes a particle of copper salt.

POTASSIUM IODIDE

Sift a portion of the feed over a sheet of white glazed paper whose entire surface has been moistened with a mixture of starch indicator and bromine water (three parts of the former to one part of the latter) in such a manner that the feed will be distributed thinly over the area of the paper.

A blue coloration denotes a particle of an iodide. If an extremely small quantity of potassium iodide is to be detected, modify the above procedure by carefully charing 10 grams or more of the feed, washing the residue with a small amount of water, and evaporating the filtered solution in a white evaporating dish so that the solids are concentrated on one small spot.

When moistened with the starch indicator and bromine water a blue coloration denotes the presence of an iodide.

Table 2 contains the collaborative results and shows the least amount of each salt that was detected in the feeds.

It is noted, in the case of ferrous sulfate, that as little as 0.00000001 per cent was detected by one analyst, 0.0000001 per cent by another, 0.000001 per cent by another and 0.0001 per cent by six others. The results ranged from 0.01 to 0.00000001 per cent.

The test for copper sulfate was less successful. Although two analysts reported their ability to detect 0.0000001 per cent, the average amount permitting identification is about 0.0001 per cent.

TABLE 2.

Minimum amount of salts detected in feeding stuffs.

ANALYST	FERROUS SULFATE	COPPER SULFATE	POTASSIUM IODIDE
	per cent	*per cent*	*per cent*
W. L. Adams Kingston, R. I.	0.05	1.0	—
J. W. Bowen	0.005	0.01	0.01
E. C. Carlyle	0.0001	0.001	0.001
J. Feugas College Station, Tex.			
Edwin G. Donohue Vermilion, S. D.	0.01	1.0	0.01
Vera Jones	0.000001	0.00001	0.001
V. White			
C. Stothers Ottawa, Can.			
W. S. Thompson Madison, Wis.	0.0001	0.0001	0.0001
Hugh J. Hennessy	0.0001	0.005	0.000001
A. P. Kerr	0.000001	0.01	—
Willis S. Hilpert	0.0001	0.0001	0.0001
W. T. Mattison	0.0004	0.0004	0.0004
George K. Redding	0.001	0.01	0.001
V. E. Munsey	0.01	—	0.01
E. L. Redfern Des Moines, Iowa	0.001	0.0001	0.00001
W. F. Walsh	0.00001	0.001	0.00001
O. B. Winters	0.00000001	0.000001	0.000001
W. G. Terrell	0.0001	0.01	0.000001
L. V. Amburgey Lexington, Ky.			
H. R. Kraybill	0.001	0.001	0.001

Potassium iodide to the extent of 0.0000001 per cent was detected by one analyst by following the modified method, and 0.000001 per cent was detected by two others. As in the case of copper sulfate, 0.0001 per cent appears to be the approximate percentage identified.

Various criticisms of the methods were made by the collaborators, the most frequent being that they were not definite or explicit. Suggestions were received in regard to the degree of fineness of the feed and the kind and size of paper. One analyst used sensitized photographic paper and submitted prints showing the colored spots produced by the reagents. Several approved the methods and desired official adoption.

In view of the fact that extremely minute quantities of each of the salts were detected and also because of the simplicity and short time required to carry out the tests, it is desirable that these methods be developed. The

adoption of some suggestions made by the collaborators and further study will aid in this accomplishment.

It is recommended—[1]

(1) That study of the Sterling method for the determination of hoof in meat by-products be discontinued for the present.

(2) That the study of the methods for the detection of traces of ferrous sulfate, copper sulfate and potassium iodide be continued during the coming year.

REPORT ON MINERAL MIXED FEEDS

By H. A. Halvorson (Department of Agriculture, Dairy, and Food, St. Paul, Minn.), *Associate Referee*

REVIEW OF PREVIOUS WORK

Before considering the results reported to the associate referee by the collaborators on mineral feeds this year, it may prove interesting to review the work done on this subject and the recommendations made heretofore. The first report on mineral feeds was read at the annual meeting of the association in 1926.[2] In this report the associate referee discussed in some detail the different classes of mineral feeds and the purposes for which they are sold. The fact that a special committee of the Association of Feed Control Officials of the United States had made a study of the subject of mineral feeds for the purpose of recommending uniform rules for labeling these products was mentioned. There was also included the recommendations of this committee for classifying mineral feeds. Since that time, however, the recommendations of this committee have been modified in some important points and have been adopted officially by the Association of American Feed Control Officials. Because of the changes and additions made in the recommendations, it is deemed advisable to repeat them here.

(a) Mixed feed containing both feed and more than 5 per cent of mineral ingredients requires, in addition to the usual declaration of the chemical feed analysis, a declaration of each ingredient contained therein and the minimum percentages of lime (CaO), phosphoric acid (P_2O_5), iodine (I), and the maximum percentage of salt (NaCl), if same is added. If minerals predominate in the mixture, the usual declaration of the chemical feed analysis, with the exception of protein, may be omitted.

(b) Mineral feeds containing no organic ingredient do not require the usual chemical feed guarantee, but do require a declaration of each ingredient contained therein and the minimum percentage of lime (CaO), phosphoric acid (P_2O_5), iodine (I), and the maximum percentage of salt (NaCl) if same are present.

(c) That the mineral ingredients be stated in the common English terms, if any such terms exist.

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 43 (1931).
[2] *This Journal*, 10, 174 (1927).

(d) It being impossible to classify separately the drug ingredients and the mineral ingredients, be it resolved:

(1) That all mixtures containing mineral ingredients generally regarded as dietary factors essential for the normal nutrition of animals and which are sold or represented for the primary purpose of supplying these minerals as additions to rations in which these same mineral factors may be deficient, be classified as mineral feeds.

(2) That all other preparations which are sold or represented primarily for the cure, mitigation or prevention of disease be classified by this association as drugs, medicines, or specifics.

The remainder of the report of the referee for that year (1926) discussed the chemical analytical problems involved in enforcing these regulations. The need was shown for suitable methods for the determination of iodine and calcium oxide in mineral products. A modified method for the determination of iodine, proposed by W. B. Griem of Madison, Wisconsin, was included in the report as was also a modified volumetric method for the determination of calcium oxide proposed by A. O. Olson of St. Paul, Minnesota. In concluding his report, the associate referee recommended (1) that the proposed method for iodine be studied and that samples be submitted to collaborators for analysis; (2) that the proposed method for lime (CaO) be studied and that samples be submitted to collaborators.

At the annual meeting of this association in 1927, the associate referee stated[1] that two samples of mineral feeds of known composition were sent to a number of collaborators with the request that calcium oxide and iodine be determined by the methods previously proposed. The results of the different collaborators are shown in tables appended to the report. The associate referee recommended (1) that the proposed method for the determination of lime (CaO) in mineral feeds be further studied and that an acetic acid modification of this method be tried for a comparison of results; (2) that the proposed method for the determination of iodine in mineral feeds published in the 1926 report be further studied and that consideration be given to other proposed methods.

In 1928[2] the recommendations made in 1927 were repeated. During 1928 and 1929 several methods for the determination of iodine in mineral feeds were submitted to the associate referee for consideration. As a result of an investigation of these methods, he gave the Knapheide-Lamb[3] method a thorough collaborative trial. The results are given for the first time in the report of the associate referee for 1929.[4] Two samples of known composition were sent to each collaborator, and determination of both iodine and calcium oxide was made on each sample. The results for iodine are shown in Table 2 of the 1929 report. In some cases they are

[1] *This Journal*, 11, 157 (1928).
[2] *Ibid.*, 12, 150 (1929).
[3] *J. Am. Chem Soc.*, 50, 2121 (1928).
[4] *This Journal*, 13, 168 (1930).

very good, but in others they are apparently disappointing. It is only justice, however, to call attention to the fact that the percentages of iodine reported by Latshaw and Coulson are several times the amounts actually present in the samples. The error resulted from the fact that the thiosulfate solution was not standardized as outlined in the method. Consideration was not given to the fact that the procedure followed develops an iodate, which in turn in the presence of potassium iodide liberates six times as much iodine as originally present. If the results reported by Latshaw and Coulson are divided by six, an average of 0.054 per cent is obtained for sample No. 1 and 0.017 per cent for sample No. 2. It is possible that collaborator Mayne R. Coe made the same error, for if his averages are divided by six, 0.0638 per cent iodine is obtained for sample No. 1 and 0.0254 per cent iodine for sample No. 2. These corrected results in both cases are much nearer to the actual amount of iodine present.

In 1929 nineteen collaborators submitted results on the determination of calcium oxide by the method proposed in 1926 and by an acetic acid modification of that method. These results are given in Table 1 of the report. The associate referee recommended: (1) that the proposed method for the determination of lime in mineral feeds be adopted as tentative and the acetic acid modification of this method be made optional, also that further work be done on this method; (2) that further work on the determination of iodine in mineral feeds be carried on by methods proposed for consideration; (3) that methods for the determination of iodine in organic minerals be studied.

Subcommittee A[1] approved Recommendations 2 and 3, but modified the first recommendation as follows: (1) That the method prepared by the referee for the determination of lime in mineral feeds be not adopted this year but that further study be made with a view to adopting it or the acetic acid modification next year, thus avoiding the adoption of two methods for the same determination.

CURRENT WORK

While it was advisable to retain the recommendation for the study of iodine in organic minerals, it was not possible, because of the large amount of work involved in the other determinations, to begin such a study this year. As a result of a canvass of chemists interested in the analysis of mineral feeds, instructions for this year's work were sent to 21 collaborators. Two samples of known composition, resembling commercial mineral feeds, were forwarded to each collaborator with directions for determining calcium oxide and iodine in both samples.

[1] *This Journal*, 13, 57 (1930).

TABLE 1.

Calcium oxide in mineral feed samples.

COLLABORATORS	SAMPLE NO. 1—32.82% CALCULATED FROM ANALYSIS OF INGREDIENTS		SAMPLE NO. 2—40.89% CALCULATED FROM ANALYSIS OF INGREDIENTS	
	Individual	Average	Individual	Average
	per cent	*per cent*	*per cent*	*per cent*
E. M. Bailey and C. E. Shepard	32.18		41.11	
New Haven, Conn.	32.04	32.06	40.97	41.11
	31.97		41.26	
J. W. Bowen	32.46		40.91	
Purina Mills,	32.44		40.95	
St. Louis, Mo.	32.49		41.15	
	32.53	32.48	41.08	41.03
	32.56		41.05	
	32.42		41.06	
M. D. Knapheide and				
W. P. Elmslie	32.79		42.33	
Moorman Mfg. Co.	32.83	32.81	42.36	42.34
Quincy, Ill.				
E. P. Greene		33.64		42.06
Tallahassee, Fla.				
W. P. Griem and L. Clifcorn	32.45		40.19	
Madison, Wis.	32.46	32.45	40.10	40.14
Kraybill and Yund	33.18		40.74	
Lafayette, Ind.	32.90		41.02	
	32.90	32.97	41.02	40.98
	32.90		41.16	
C. S. Ladd and W. L. Roberts	32.50		41.02	
Bismarck, N. D.	32.32		41.23	
	32.36		41.09	
	32.57	32.42	41.02	41.12
	32.40		41.23	
	32.36		41.16	
V. E. Munsey	32.35		41.66	
Washington, D.C.	32.49	32.40	41.86	41.86
	32.35		42.07	
A. O. Olson	32.63		41.34	
St. Paul, Minn.	32.61		41.34	41.34
	32.62	32.62		
	32.61			
W. F. Hand and H. Solomon		32.35		41.56
A. & M. College, Miss.				

Sample No. 1 consisted of the following ingredients and proportions: tankage 10 per cent, charcoal 10 per cent, spent bone black 25 per cent, ground limestone 35 per cent, salt (sodium chloride C.P.) 19.9 per cent, potassium iodide 0.1 per cent. Determinations of calcium oxide in each ingredient by the method being studied this year showed that the tankage furnished to the mixture 0.93 per cent, the charcoal 0.42 per cent, the spent bone black 11.98 per cent, and the limestone 19.49 per cent, making a total of 32.82 per cent. Assuming that the method used gives accurate results and that the individual determinations are correct and also that there is no calcium oxide in the salt and the potassium iodide, the amount of calcium oxide in sample No. 1 should be 32.82 per cent. Since there was added to the mixture represented by sample No. 1 exactly 0.1 per cent potassium iodide, it will be found from calculation that this sample contains 0.0764 per cent of iodine.

Sample No. 2 consisted of these ingredients: 40 per cent tri-basic calcium phosphate (pure precipitated), 40 per cent calcium carbonate (precipitated), 19.95 per cent salt (sodium chloride C.P.), and 0.05 per cent potassium iodide. Determinations of calcium oxide in the manner described in the preparation of sample No. 1 showed that the calcium phosphate furnished to this mixture 18.87 per cent, and the calcium carbonate 22.02 per cent, making a total of 40.89 per cent calcium oxide in sample No. 2. From the percentage of potassium iodide used in this mixture, it will be seen from calculation that sample No. 2 contained 0.0382 per cent iodine.

The instructions sent to the collaborators contained information on the care and the accuracy with which the samples had been prepared. It was stated that although sample No. 1 did not appear uniform on account of the tendency of the ingredients to segregate, care had been exercised to insure that each lot, as a whole, contained the correct proportions of all the ingredients. Sample No. 1 was characteristic of many commercial mineral feeds found on the market and was different from previous A.O.A.C. samples of mineral feeds in that charcoal had been used as an ingredient. Collaborators were advised to thoroughly mix sample No. 1 and grind all the material fine enough to pass either a 30-mesh sieve or the sieve recommended in the official methods. It was pointed out that after grinding and before weighing each portion for analysis, it was advisable to empty the entire contents of the bottle on a paper or mixing cloth. After being thoroughly mixed, the sample for weighing could be taken with a spatula at various places in the pile. The same directions for mixing and weighing were given in connection with sample No. 2, but the condition of this sample did not make grinding necessary.

The collaborators were requested to determine calcium oxide or lime (CaO) in both samples by the method published in the report of the associate referee for 1926. The results of these determinations are shown in

Table 1. Many of the collaborators expressed satisfaction with the convenience and fair degree of accuracy of this method, and some suggested further improvement. As the acetic acid modification of this method did not offer any particular advantages over the method as originally written, it was decided to recommend tentative adoption of the method as published.

The results submitted by the collaborators on the iodine determinations by the Knapheide and Lamb method this year are much more encouraging than they were last year. It is evident now that any analyst who has had sufficient experience with this method can obtain fairly good results on mineral feeds. In answering a questionnaire this year the collaborators indicated that the most probable reason for erratic results was the use of too much potassium nitrate. The authors state that this reagent is necessary in samples containing large amounts of charcoal, but that 10 grams is excessive in samples containing little or no charcoal. All the collaborators from whom answers were received stated that last year they had used 10 grams of potassium nitrate for each sample. Sample No. 2 contained neither charcoal nor tankage, but sample No. 1 last year contained 10 per cent tankage.

The authors of the method have now added this precaution: "Ten grams of potassium nitrate are added before fusion if the sample contains from 15–20 per cent charcoal or organic material. If no charcoal or organic material is present, no potassium nitrate should be added before fusion and only a few small crystals at the end. Potassium nitrate should be added according to the amount of charcoal or organic material present and care should be used to avoid a large excess of this reagent."

In the last paragraph on page 2123 of the method,[1] substitute "30 minutes" for "20 minutes" and "350 cc." for "400–500 cc." Also, substitute "6 minutes longer" for "5 minutes longer." At the top of page 2124, insert the words "or 1 cc. of phosphoric acid 85 per cent" after the words "5 cc. of 20 per cent." In the last paragraph, the word "should" is to be substituted for "may."

From the experiences of a number of the collaborators, it is evident that the use of a suitable furnace for making the fusions is of considerable help in obtaining satisfactory results. Specifications for a suitable furnace for making the fusions are as follows:

Use a sheet iron cylinder 4 inches wide and 12 inches long, the top to have an opening in the center large enough to accommodate a 100 cc. nickel crucible. Suspend a 2¾ inch circular plate in the center of the cylinder 3 inches below the top for spreading the flame, thereby preventing the free flame from coming in contact with the crucible and providing uniform heat. Make a slot at the bottom of the cylinder 1 inch wide by 3 inches high for admitting air and the burner tubing, and near the top rim make eight ¼ inch holes to allow for the escape of the exhaust gases.

[1] *J. Am. Chem. Soc.*, **50**, 2121 (1928).

With this type of crucible furnace much better oxidation reaction is obtained within the crucible than when it is heated over a free flame or on a wire gauze.

Other precautions which were sent to the collaborators both in 1929 and 1930 follow; they are given here because they were not a part of the method as originally published.

TABLE 2.

Iodine in mineral feed samples.

COLLABORATORS	SAMPLE NO. 1—0.0764% ADDED		SAMPLE NO 2—0 0382% ADDED	
	Individual	Average	Individual	Average
	per cent	*per cent*	*per cent*	*per cent*
E. M. Bailey and W. T. Mathis	0.0768		0.0348	
	0.0735	0.0743	0.0309	0.0324
	0.0727		0.0314	
E. M. Bailey and C. E. Shepard	0.0701	0.0701	0.0273	0.0273
J. W. Bowen	0.0717		0.0359	
	0.0695		0.0362	
	0.0695	0.0718	0.0316	0.0346
	0.0765			
M. D. Knapheide and W. P. Elmslie	0.0790		0.0383	
	0.0758	0.0779	0.0377	0.0375
	0.0790		0.0365	
W. B. Griem and L. Clifcorn	0.0727		0.0295	
	0.0715	0.0721	0.0364	0.0330
	0.0721		0.0331	
C. S. Ladd and W. L. Roberts	0 0749		0 0395	
	0.0743		0.0400	
	0.0779		0 0385	
	0.0733	0.0750	0.0394	0.0392
	0.0761		0.0390	
	0.0735		0.0389	
V. E. Munsey	0.0623		0.0276	
	0.0617	0.0620	0.0268	0.0272
A. O. Olson	0.0768		0.0193	
	0.0732		0.0342	
	0.0674	0.0712	0.0344	0.0328
	0.0712		0.0388	
	0.0674		0.0374	
W. F. Hand and H. Solomon		0.0669		0.0328

The oxidation reaction must not be allowed to proceed too rapidly. It can be retarded by dipping the bottom of the crucible in water.

The addition of phosphoric acid must be rapid in order to avoid running over the end point as the methyl orange is rapidly destroyed in an acid solution under the conditions that exist in this determination. A large excess of acid will cause low results.

The fusion should be handled carefully, and it is necessary to boil the solution long enough to get rid of *all* the sulfurous acid as well as to remove *all* the bromine at a later stage.

A review of the comments of the collaborators on the Knapheide and Lamb method for iodine in mineral feeds shows them to be similar to ones received last year. While most of the collaborators complained about the tediousness of the method, no other dissatisfaction with it or the results obtained were indicated. The collaborative results for this year are shown in Table 2. A study of these results indicates that analysts who have had some experience with the method can determine iodine in simple or complex mineral feeds with a reasonable degree of accuracy.

RECOMMENDATIONS[1]

It is recommended—

(1) That the proposed method for the determination of lime in mineral feeds be adopted as tentative and that additional work be carried on next year.

(2) That further work on the determination of iodine in mineral feeds be carried on by the Knapheide and Lamb method and other methods proposed for consideration.

(3) That methods for the determination of iodine in organic minerals be studied.

REPORT ON MOISTURE IN FEEDING STUFFS

By G. E. GRATTAN (Department of Agriculture, Ottawa, Canada), *Associate Referee*

The associate referee continued the investigation on moisture determination in feeding stuffs. Four samples were sent out to several laboratories for collaborative work; results were received from three. The method is as follows:

Regulate an air oven to 135°C. ± 2°. Using low, covered, aluminum dishes, weigh approximately 5 grams of the sample into each dish and shake lightly until the contents are evenly distributed. With the covers removed, place the dishes and covers in the oven as quickly as possible and dry the samples for 2 hours. Place the covers on the dishes and transfer them to a desiccator to cool. Weigh, and calculate the loss in weight as moisture.

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 43 (1931).

It was requested that moisture be determined by the vacuum oven method for comparison and also that the drying be discontinued in the 135°C. air oven at the end of 1 hour.

Collaborative results.

LABORATORY	BRAN	MOLASSES FEED	OILCAKE MEAL	FISH MEAL
	Official vacuum method.			
	per cent	*per cent*	*per cent*	*per cent*
1	7.9	6.1*	8.0	6.1
2	8.2	7.3	8.3	—
3	8.3	7.1	8.5	6.4
Average	8.1	7.2	8.3	6.3
	Electric air oven, 1 hour at 135°C.			
1	6.5	7.0	6.5	5.8
2	8.0	7.9	8.3	—
3	8.0	7.5	8.4	6.4
4	7.1	8.0	7.5	6.6
	Electric air oven, 2 hours at 135°C.			
1	7.8	8.9	8.2	6.1
2	8.5	8.5	8.1	—
3	8.5	8.7	8 4	6.9
4	7.6	8.6	8.1	6.7
Average	8.1	8.7	8 2	6.6
Mean difference between official vacuum method average and the average for air oven at 135°C. for 2 hours.				
	0.0	1.5	0.1	0.3

* Dried at 60°C. in vacuum oven.

REMARKS

With the exception of molasses feed, the results would appear to be well within the tolerance of experimental error.

It would appear that this method could not be used for the determination of moisture in feeds containing an appreciable amount of molasses or sugars.

The samples were examined under the microscope after drying at 135°C. for two hours. It was found that the molasses feed had become somewhat caramelized. The floury portion in the bran was a shade darker than before it was heated. The oilcake meal was unchanged, and the fish meal was slightly darker. It would appear that heating cereal products, meat products and oilcake meals for two hours at 135°C. has little chemical effect on them, but that feeds containing sugars, etc., undergo decomposition.

It is recommended[1] that the electric air oven method for the determination of moisture in feeding stuffs that do not contain sugars be adopted as official (first action).

REPORT ON SUGARS AND SUGAR PRODUCTS

By R. T. BALCH (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

This year's work on sugars and sugar products will be represented by the detailed reports of the Associate Referees on Honey; on Maple Products; on Drying, Densimetric and Refractometric Methods; on Polariscopic Methods and on Chemical Methods for Reducing Sugars. There will be no report on starch conversion products because this associate refereeship has remained unfilled. No attempt will be made here to review these reports, but it is desirable to state that these contributions should prove very valuable, not only to the association but to all engaged in analytical work. The general referee approves of the conclusions reached and of the recommendations proposed by the associate referees.

In addition to supervising collaborative work on maple products and on polariscopic methods, the general referee gave much thought and time during the year to the revision of the chapter "Sugars and Sugar Products" in *Methods of Analysis*. The changes recommended were made the subject matter of a separate report which will be considered later.

REPORT ON HONEY

NELSON'S MODIFICATION OF THE FIEHE TEST FOR THE DETECTION OF ARTIFICIAL INVERT SUGAR

By H. A. SCHUETTE[2] (Department of Chemistry, University of Wisconsin, Madison, Wis.), *Associate Referee*

Studies centering around the qualitative detection of artificial invert sugar in honey have been intermittently before this association since 1914, when Shannon[3] reported on the results of a critical survey of nine of the most common tests for this adulterant. Out of this and subsequent studies[4] there eventually resulted the tentative adoption of a modified form of Fiehe's resorcinol[5] test and the aniline chloride procedure[6] of Feder; the former is applicable to all types of honey, but the latter is limited to the examination of light colored samples only. It is a characteristic of both tests that in spite of detailed description, considerable experience is required on the operator's part in order to interpret correctly the results of

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 43 (1931).
[2] Presented by R. T. Balch.
[3] *This Journal*, 1, 472 (1915).
[4] Shannon, *This Journal*, 2, 169 (1916); Sherwood, *ibid.*, 5, 429 (1921); 7, 345 (1923); Seaman, *ibid.*, 8, 364 (1925).
[5] *Ibid.*, 22, 412 (1911).
[6] *Z. Nahr. Genussm.*, 15, 492 (1908); 16, 75 (1909).

these tests. Furthermore, in the light of present knowledge[1] a positive Fiehe test may be considered as conclusive evidence of the presence of commercial invert sugar in honey only when the latter has not been stored for some length of time after having been heated to temperatures of approximately 72°C.; yet, conversely, when both tests are negative this fact is not necessarily regarded as indicative of the absence of this adulterant.

Because of the difficulties inherent in both the original and the modified Fiehe procedures[2] with respect to a thorough extraction of that fructose decomposition product, oxymethylfurfural,[3] which is responsible for the color reaction with resorcinol or aniline chloride, and a separation of the stubborn and persistent emulsion which sometimes forms, Nelson[4] proposed a modification in the technic of this test, the salient feature of which is the use of a device which makes possible the continuous extraction of a liquid with an immiscible solvent. His method follows:

Dissolve 2 grams of honey in 10 cc. of water, and extract the solution rapidly with ether in a Palkin-Watkins[5] extractor for 30 minutes. Concentrate the ether to about 5 cc. and transfer to a test tube. Add 2 cc. of resorcin reagent, freshly prepared by dissolving 0.2 gram of resorcin in 20 cc. of concentrated hydrochloric acid, and shake the mixture immediately. Note the colors at the end of 5 minutes.

Inasmuch as the substitution of the novel features of this procedure for the present preliminary steps incident to the Fiehe test seemed to offer promise of simplifying it, the whole question of the usefulness of this procedure as a qualitative test for artificial invert sugar in honey was reopened and made the subject of collaborative study. To that end four official samples, representative of the light and dark types, were prepared and sent out, together with an extractor made from an 8-inch Pyrex test tube, at whose middle section there had been sealed a delivery tube of 5/16 inch internal diameter bent downwards at a right angle. The inner tube was of the same diameter. A description of the experimental samples follows:

TABLE 1.

Description of experimental honeys.

SAMPLE	PREDOMINANT FLORAL SOURCE	REMARKS
A	Spanish needle	Strained from the comb without heating
B	Clover	An extracted commercial sample
C	Clover	Contained 10% commercial invert sugar sirup
D	Clover	Contained 20% commercial invert sugar sirup

[1] *This Journal*, **8**, 260 (1925).
[2] Bryan, U. S. Dept. Agr. Bur. Chem Bull. **154**, p. 15; *Methods of Analysis, A O A C*, 1925, 201
[3] Kaiser *Arb. kais. Gesundh.*, **30**, 637 (1909); *Z. Nahr. Genussm.*, **18**, 331 (1909), von Eckenstein and Blanksma, *ibid*, **19**, 346 (1910).
[4] *This Journal*, **12**, 323 (1929).
[5] Palkin, Murray and Watkins, *Ind. Eng Chem.*, **17**, 612 (1925).

Invitation to participate as collaborators in this study was accepted by W. L. Roberts of the State Regulatory Laboratory, Bismarck, N. D.; Thomas A. Balthis, Division of Chemistry, Department of Agriculture and Immigration, Richmond, Va.; E. O. Huebner, Department of Agriculture and Markets, Madison, Wis.; J. B. Wilson, U. S. Food and Drug Administration, Washington, D. C.; and Howard C. Hansen, a student in the Department of Chemistry, University of Wisconsin. Of this number four submitted reports of their analyses.

The collaborators were asked (1) to test each sample for commercial invert sugar by the modified method of Fiehe; (2) to repeat the test, this time following the suggestion of Nelson as given in preceding paragraphs; and (3) to re-extract those samples once treated by the Nelson method. As a guide in formulating reports, it was suggested that the following questions be answered:

1. Did the dark honeys yield a colored extract?
2. Disregarding a yellow or salmon shade of color as having no significance, which samples contain artificial invert sugar?
3. Does the extraction of the oxymethylfurfural appear to be complete, or substantially so, in 30 minutes?
4. What is your judgment as to the value of this modified procedure?

The results reported by the several collaborators, when summarized in tabular form (Table 2), show that a majority reached the same conclu-

TABLE 2.

Results of collaborative study of the Nelson modification of the Fiehe test.

SAMPLE	ROBERTS			HUEBNER			WILSON			HANSEN		
	1	2	3*	1	2	3	1	2	3	1	2	3
A	+	−	−	−	−		?	?	−		−	
B	−	−	−	−	−		−	+	−		−	
C	+	+	−	−	+	?	+	−	−		+	
D	+	+	−	+	+	−	+	+	?		+	

* 1. Bryan's modification, or present tentative A O.A C. test.
2. Nelson's modification
3. Second extraction in Nelson's test.

sions. Since only two indicated the color phenomena on which they based their conclusions, this feature has not been included in this summary. All reported that they had obtained an uncolored ether extract and that re-extraction of the sample served no useful purpose.

COMMENTS OF COLLABORATORS

W. L. Roberts.—The extraction of oxymethylfurfural is substantially complete in 30 minutes. I prefer the modified procedure, because the color reaction is easier to detect and there is no trouble with emulsions. Moreover, the Fiehe test as given in *Methods of Analysis* does not give a color immediately after shaking.

Roberts also commented on the fact that if cognizance is taken of the *immediate effects* produced when the resorcinol reagent is added as directed in the present tentative Fiehe test, the absence of oxymethylfurfural must be assumed in all the samples. His conclusions, however, were based upon observations made 5 minutes after the addition of the reagent.

E. O. Huebner.—The technic employed in the Nelson modification is obviously superior to the A.O.A.C. tentative method for extracting oxymethylfurfural from honey. From the limited experience I have had with both procedures I am of the opinion that the test for commercial invert sugar is more reliable when the Nelson modification of the Fiehe test is used.

Huebner re-extracted only one sample and found then that the resorcinol reaction was negative.

J. B. Wilson.—Commenting on the A.O.A.C. method, tests were unsatisfactory as colors did not develop immediately. Separation of ether was difficult because of emulsification.

Wilson found the proposed modification more satisfactory than the present procedure. He reported an extraction rate of about 200 drops per minute, but he did not use the extractor sent out by the associate referee.

H. C. Hansen.—The modified technic of Nelson appeals to me as an improvement over the current procedure. After the sample had been treated by the latter method, a long time was required for the ether to separate out in a clear layer, and then separation was accomplished only after centrifugal action had been used. No such difficulty was experienced when the newer procedure was used.

Hansen advanced the opinion that the rate and time of extraction should be increased when the analyst is extracting a sample suspected of containing a large amount of adulterant.

DISCUSSION

Although unanimity of opinion as to the presence of hydroxymethylfurfural in the four honey samples did not prevail among the collaborators, the results obtained by embodying Nelson's technic with the current practice raises the thought that this modification bids fair to become a useful tool in the simplication of a test in which, to some extent, the personal equation of the operator is inherent. One collaborator was in doubt about the interpretation of the reaction obtained with the sample containing 10 per cent of adulterant, although he reported a negative test when he examined this sample by the present method. Another analyst appears to have been more successful in his interpretation of the reactions obtained by the original procedure than with the modification, although he expressed a preference for the latter. A third collaborator was so successful in his manipulation of the Nelson modification as to venture the opinion that the approximate ratio of the adulterants present was as 1:2, which is correct. Finally, the fourth member of the group appears to have

found no difficulty in correctly diagnosing the tests obtained by this same procedure.

RECOMMENDATIONS[1]

It is recommended—

(1) That steps be taken to modify the present tentative method of Fiehe in conformity with the change in technic suggested by Nelson.

(2) That the present tentative aniline chloride reaction of Feder be studied with a view to adapting Nelson's procedure to it, with the ultimate purpose of dropping it as a separate test as it now stands and thus unifying the association's methods for the qualitative detection of oxymethylfurfural.

(3) That Nelson's method be further studied as to its delicacy with respect to honeys containing less than 10 per cent of commercial invert sugar.

(4) That the unfinished studies of the association pertinent to honeys heated as in commercial practice and then stored for various lengths of time be resumed along lines embodied in the foregoing recommendations.

Some thought might also be given to the initiation of studies relevant to the diastatic activity of honey.

REPORT ON MAPLE PRODUCTS[2]

By J. F. SNELL (Macdonald College, Province of Quebec, Canada), *Associate Referee*

Collaborative work was continued this year on the preparation of samples for analysis and on electrical conductivity and various forms of the Canadian lead method.

Table 1 indicates the parts taken in the work by the eleven collaborators. Table 2 describes the 24 pure, and Table 3 the six adulterated sirups used. All sirups were of Quebec Province origin; Nos. 25–27 were made in 1929, the others in 1930.

The adulterated samples were made by adding to pure sirups suitable volumes of a sirup made from commercial refined white sugar. This sirup contained about 66 per cent of solids. The approximate percentages of cane product in the six adulterated sirups are shown in Table 3.

Before shipment to collaborators and before the corks were put in, the bottled samples were sterilized by heating to 70°–80°C. for 1 hour in a water bath.

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 43 (1931).

[2] In the preparation of this report substantial support was received from the National Research Council of Canada under a grant to the Associate Referee for research on maple products. By kind permission of the Minister of Agriculture for the Province of Quebec assistance in the collection of samples was afforded by the local Agriculturists (Agronomes) of the Department.

TABLE 1.

Collaborators on maple products, 1930.

REFERENCE LETTER	LABORATORY	ADDRESS	DIRECTOR	ANALYST	NUMBER OF SAMPLES ANALYZED FOR—						
					Total Solids as		Conductivity Value		Lead Values		
					Rec'd	Prep'd	22% solids	25% solids	Tentative	Fowler	1 cc.
A	Macdonald College	Macdonald College, P.Q., Can.	J. F. Snell	H. J. Atkinson	30	30	27	30	30	30	30
S	do	do	do	Lev Skazin	20	20	20	20	20	20	20
F	do	do	do	G. H. Findlay	10	10	10	10	10	10	10
K	Penick & Ford	Cedar Rapids, Iowa	C. C. Kesler	C. C. Kesler	30	30	30	30	30	30	30
B	Chem. & Tech. Research, U. S. Bur. Chem. & Soils	Washington, D.C.	R. T. Balch	S. Byall	30	30	—	—	30	30	30
C	Amer. Tobacco Co.	Richmond, Va.	A. L. Chesley	H. R. Hanmer	30	27*	—	—	20	20	20
H	Acadia University	Wolfville, N.S., Can.	D. U. Hill	D. U. Hill	15	15	15	15	15	15	15
T	Quebec Official	St. Hyacinthe, P.Q. Can.	J. E. Theriault	J. E. Theriault	30	30	—	—	30	—	30
R	U.S. Food and Drug Adm.	Chicago, Ill.	H. Runkel	E. H. Berry	9	9	—	—	9	9	9
M	N.Y. Sugar Trade	New York, N.Y.	F. W. Zerban	J. E. Mull	30	—	30	30	—	—	—
G	do	do	do	C. A. Gamble	—	4	—	—	—	—	—

* Results of Analyst C on 7 sirups as prepared are omitted from Table 4.

TABLE 2.

Pure maple sirups used in collaborative experiments, 1930.

NUMBER	MAKER	ADDRESS	COUNTY	RUN	
1	Parker	Hatley	Stanstead	Last	
4				First	
7	Filion	Lachute	Argenteuil	Last	
8				Middle	
9				First	
10	Kerr	Lakefield	Argenteuil	Last	
11				Middle	
12				First	
13	Rodger Bros.	Lachute	Argenteuil	Last	
14				Middle	
15				First	
16	Halliday	Sawyerville	Compton	Last	
17				Middle	
18				First	
19	F. Poulin	St. Benoit	Beauce	Last	
20				Middle	
21				First	
22	E. Poulin	St. Martin	Beauce	Late	
23				Middle	
24				Early	
25	Bannister	Knowlton	Brome	Late	1929
26				Middle	1929
27				First	1929
28	Mixture, consisting mainly of Nos. 22–27.				

TABLE 3.

Adulterated sirups used in collaborative experiments, 1930.

NUMBER	MADE FROM PURE SIRUP NUMBER	CANE SUGAR SIRUP
		per cent
2	1	20
3	1	57
5	4	20
6	4	40
29	28	20
30	28	50

PREPARATION OF SAMPLE

The work done in 1929 having shown the existent official method of preparation to be unreliable, collaborators were instructed to prepare sirups by the following proposed method and to comment thereon:

PREPARATION OF SAMPLE—PROPOSED METHOD

Transfer approximately 100 cc. of a uniform mixture of the sample, including any sediment, to a casserole or beaker; add one-fourth the volume of water and evaporate over a flame. When the temperature of the boiling sirup approaches 104°C., draw a small quantity into a thin-walled pipet of about 1 cc. capacity and cool to room temperature in running water. Wipe the outside of the pipet, allow the diluted sirup in the point to escape, and make a refractometric measurement of the solids content of the cooled sirup. Repeat this operation from time to time until a reading corresponding to 64.5 per cent solids ($n^{20} = 1.4521$) is obtained. Pour all the sirup onto a 19 cm. extra rapid filter in a hot-water-jacketed funnel, cover with a watch-glass, receive the filtrate in a narrow-mouthed bottle, and stopper immediately.

Analyst S, who prepared the samples for distribution to the collaborators, obtained in the 24 pure sirups as received from the makers refractometric dry matter results varying from 61.59 to 70.69 and averaging 65.71 per cent.

Dry matter results on the samples as received by the collaborators might be expected to be a little higher than those on the sirups before sterilization. The results of S on the 16 pure sirups analyzed by him were actually higher by 0.03 to 0.99 per cent (average 0.50 per cent). Those of the collaborators in general, although averaging slightly higher (66.10 as against 65.71 per cent) showed such wide variations among themselves as to render this comparison of averages of little value. Among the 30 sirups were one upon which the maximum and minimum values reported differed by 5.04 per cent, 2 by between 4 and 5 per cent, 2 by between 3 and 4, 1 by between 2 and 3, 3 by between 1.5 and 2, and 6 by between 1.0 and 1.5 per cent. On only one sample was the range of results less than 0.5 per cent. The remaining 14 showed variations of between 0.5 and 1.0 per cent.

The differences of 1.0 per cent or less shown in half the samples are not greater than might be expected. Furthermore, the omission of one extreme in each of the other samples would bring all but one inside the limit of 1.0 per cent. There is also some indication that the extreme errors may be personal or due to variations in the instruments in the different laboratories. The minimum reading in 15 of the sirups was obtained by one analyst (who was also second lowest in 11 other samples) and the maximum in 12 of the sirups by another, while two other analysts were responsible, respectively, for 5 maximum and 8 minimum and for 5 maximum and 3 minimum readings. Analysts A and S made readings on sirups 1–20 and analysts A and F on sirups 21–30 on one instrument, obtaining results within 0.5 per cent except in three instances. In one of these the difference

was only 0.59. In the others the analyst other than A obtained a result quite out of line with those obtained by any of the other collaborators (in one instance high, in the other low)—a circumstance which suggests possible misreading or accidental contamination of the sample. In view of these results further work in the comparison of refractometric readings obtained in different laboratories is to be recommended.

Refractometric dry matter results upon the samples after preparation for analysis were also reported by each analyst. The purposes of the dilution and reconcentration involved in the process of preparation are: (1) to bring all samples to the standard water content of 35 per cent; (2) to redissolve substances which may have been forced out of solution by overconcentration in manufacture or by conversion into maple sugar; (3) to force out of solution any excess substances left dissolved on account of under-concentration in manufacture.

Several analysts found the particular filter paper recommended in the original instructions unsatisfactory, passage of 100 cc. of sirup consuming an hour or more. Although papers permitting passage of that volume of sirup in 5 minutes or less were subsequently found and recommended the results reported included many obtained with the slower filters. There can, therefore, be little doubt that the method is capable of giving closer results than were obtained.

As they stand, the results show a vast improvement over those obtained last year with the official method. The average moisture content attained by eight analysts on from 9 to 30 sirups varies only from 65.31 to 66.63, as compared with last year's range of 57.4 to 65.3 per cent on 20 sirups by 10 analysts, only two of whom came near to the goal of 65.0 per cent. The greatest variation in the solids content of sirups prepared by an individual this year was 5.97 and the next greatest 3.3, as against 11.5 and 9.6 last year. One of this year's collaborators (F) found the solids of all his 10 prepared sirups to fall within a range of 0.71 per cent, another (C) reported 20 with a range of 1.11 per cent but afterwards in preparing 7 of the remaining 10 (not included in the table) extended his range to 1.68. The average difference between the maximum and the minimum of the eight analysts is 2.58. The corresponding figure for the official method last year was 7.7.

Of the 164 preparations made by the collaborators only 31 (19 per cent) fell below 65.0, of which only 12 were below 64.5 and only 2 below 64.0. The general average of the 164 was 65.6; that for 202 preparations by 10 analysts last year by the official method was 61.6.

Allowing for error in the refractometer reading and for further error due to the actual but avoidable slowness of filtration encountered by some collaborators, the proposed method must be regarded as far superior to the official method. With some variation in the directions it may be recommended for substitution for the latter on a tentative basis. As amended

it should receive further study with reference to suggestions made by the collaborators.

One of these suggestions is to concentrate beyond 65.0 per cent solids, filter, and adjust back to 65.0 by addition of water. Provided the over-concentration were not carried so far as to force any calcium malate out of solution and that the adjusting water were thoroughly incorporated into the sirup the suggestion might be accepted. Such procedure would have the advantage of bringing the quantities to be weighed for dilution for the determination of lead and conductivity values to nearly the same value for all sirups and of even rendering possible the use of a fixed weight or volume of sirup.

The Referee on Sugars and Sugar Products suggested the use of a kieselguhr filter-aid and filtration with suction as more convenient than the use of a hot water funnel. The only objection to vacuum filtration is that it tends to increase the evaporation of the sirup and so to cause over-concentration, and this objection might not be serious if the filtration were sufficiently rapid and adjustment to 65.0 per cent were adopted. Indeed, it is not impossible that with sufficiently rapid filter paper, and perhaps a filter-aid, even filtration at atmospheric pressure can be rendered sufficiently rapid to make the use of a hot water jacket unnecessary.

LEAD NUMBERS

The lead number work was a continuation of last year's and consisted in comparisons of the tentative Canadian method, the Fowler modification thereof and the modification of the latter in which 1.0 cc. of reagent is used instead of 2.0 cc. To save labor, work with 1.5 cc. was omitted.

The basic lead acetate solutions used by nine collaborators were analyzed by Analyst F for:

A. *p*H value by diluting 2 cc. of the solutions to 40 cc., adding 0.5 cc. of phenol red solution and comparing the color with a set of La Motte standards, which were checked by use of buffered solutions prepared from acid potassium phosphate. Wherever the *p*H fell within the range of cresol red, check determinations were made with that indicator.

B. Alkalinity and total lead and, by derivation, neutral and basic lead, according to the methods described in the report for 1920.[1]

The results of the analysis of these solutions, together with the average lead value obtained by each analyst with his own solution upon groups of samples of the sirups, pure and adulterated, are given in Table 4. At his own suggestion the lead values found by Analyst C are separated from the others on account of the exceptional conditions under which they were determined. One of his determinations on each sample by each method was made while the room temperature was about 100°F. Checks were made10

[1] *This Journal*, 4, 430 (1921).

TABLE 4.

Basic lead acetate solutions and average lead values.

DETERMINATION	B	A	K	T	S	H	R	F	MEAN	C
*p*H value	7.1	7.4	7.5	7.2	7.3	7.2	7.1	7.45	7.28	7.3
Alkalinity (cc. 0.1*N* acid per cc.)	6.69	9.465	10.14	6.735	9.065	7.74	6.51	9.31	8.207	9.235
Lead per cc.:										
Total	0.2211	0.2294	0.2257	0.1991	0.2301	0.2397	0.2029	0.2201	0.2210	0.2302
Neutral (by diff.)	0.1518	0.1314	0.1207	0.1294	0.1362	0.1595	0.1355	0.1237	0.1360	0.1346
Basic	0.0693	0.0980	0.1050	0.0697	0.0939	0.0802	0.0674	0.0964	0.0850	0.0956
Ratio $\frac{\text{Neutral}}{\text{Basic}}$	2.19	1.34	1.14	1.85	1.45	1.98	2.01	1.28	1.6	1.40
Average Lead Values:										
Tentative Method										
Samples 1–30	3.01	3.43	3.28	3.66					3.35	
" 1–20	3.02	3.43	3.21	3.64	3.06				3.27	4.16
" 1–10	2.93	3.32	2.96	3.49	2.73	2.88	2.76		3.01	3.86
" 20–30	3.01	3.42	3.42	3.69				3.41	3.39	
Fowler Method										
Samples 1–30	3.14	3.60	3.68						3.47	
" 1–20	3.16	3.58	3.64		3.36				3.44	4.18
" 1–10	3.00	3.45	3.35		3.07	3.17	2.90		3.16	4.04
" 20–30	3.10	3.63	3.76					3.68	3.54	
Proposed Method (1 cc.)										
Samples 1–30	3.01	3.53	3.72	3.27					3.38	
" 1–20	3.03	3.52	3.68	3.29	3.53				3.41	4.14
" 1–10	2.92	3.41	3.42	3.16	3.21	2.96	2.78		3.12	4.00
" 20–30	2.97	3.55	3.78	3.25				3.62	3.43	

TABLE 5.

Lead values. Comparison of methods. Range of value in genuine sirups, 1930.

ANALYST:	B	A	K	T	S	H	R	F	C
Samples analyzed, Nos.	1–30	1–30	1–30	1–30	1–20	1–15	1–9	21–30	1–20
Pure sirups analyzed	24	24	24	24	16	11	5	6	16
Tentative Method:									
Maximum in No.	1	1	23	1	19	1	1	23	19
Minimum in No.	27	4	4	4	4	4	4	27	4
Average	3.24	3.75	3.59	3.93	3.36	3.37	3.48	3.76	4.50
Maximum	4.81	5.31	4.88	4.76	4.93	4.68	4.55	4.45	6.52
Minimum	2.23	2.71	2.49	2.77	2.20	2.10	2.23	2.71	3.03
Range	2.58	2.60	2.39	1.99	2.73	2.58	2.32	1.74	3.49
Range % of Minimum	116	96	96	72	124	123	104	64	115
Range % of Average	80	69	67	51	81	77	67	46	54
Fowler Method:									
Maximum in No.	1	1	19		1	1	1	23	8
Minimum in No.	27	27	4		4	4	4	27	4
Average	3.54	3.92	3.90		3.65	3.50	3.29	4.04	4.51
Maximum	4.87	5.34	5.43		5.47	4.68	4.46	4.65	5.94
Minimum	2.32	2.87	3.05		2.52	2.37	2.91	2.90	2.98
Range	2.55	2.47	2.38		2.95	2.31	1.55	1.75	2.96
Range % of Minimum	110	86	78		117	98	53	60	99
Range % of Average	72	63	61		81	66	47	43	66
Proposed Method:									
Maximum in No.	1	1	23	9	1	9	1	23	1
Minimum in No.	27	4	4	4	4	4	4	27	4
Average	3.19	3.79	3.97	3.46	3.79	3.36	3.29	3.93	4.43
Maximum	3.97	4.64	4.82	3.95	4.82	4.09	3.67	4.65	5.57
Minimum	2.28	2.98	3.23	2.85	2.85	2.57	2.83	3.12	3.30
Range	1.69	1.66	1.59	1.10	1.97	1.52	0.84	1.53	2.27
Range % of Minimum	74	56	49	37	69	59	30	49	69
Range % of Average	53	44	40	32	52	45	26	39	51

days later at a room temperature of about 85°F., after the samples had suffered more or less deterioration by growth of molds. In the majority of instances the checks showed fair agreement with the previous determinations; in a few instances there was a marked divergence. This analyst's results on all the sirups were higher than those of other collaborators, though his lead solution appears from the analysis to be very similar to that of Analyst S.

In Table 5 are summarized the average, the maximum and the minimum results obtained by each analyst upon the *genuine* sirups analyzed by him. The range between maximum and minimum is expressed as percentage of the minimum as well as of the average.

Table 6 exhibits the range of the Canadian lead values as determined by the various methods in 47 genuine sirups, including those used in the collaborative work of 1929 and 1930 and three previously reported by

TABLE 6.

*Range of lead values in genuine sirups examined in 1929 and 1930 and 3 others.**

METHOD	NUMBER OF SIRUPS	MAXIMUM	MINIMUM	RANGE	RANGE PERCENTAGE OF MINIMUM
Tentative, 2 cc. Hot washing	47	6.16	2.02	4.14	205
(Tentative including C's results	47	6.52	2.02	4.50	223)
Tentative, including earlier analyses†	192	7.55	1.74	5.81	334
Fowler, 2 cc. Cold washing	47	6.37	2.32	4.05	174
1.5 cc. Cold washing	20	6.10	2.54	3.56	140
1.0 cc. Cold washing	47	5.47	2.28	3.19	140

* Fowler and Snell, *Ind. Eng. Chem.*, Anal Ed , 1, 11 (1929).
† Snell, Trans Roy Soc Canada, sec. III, p. 228 (1919)

Fowler and Snell. The range of the "tentative" value as previously found in 155 genuine sirups in the Macdonald College laboratory extends in both directions beyond that of these recent analyses and consequently represents the extreme range found in 192 samples. The range of the Fowler value is materially less than that of the Canadian lead value as determined by the tentative method The range of the 1.0 cc. value is still narrower.

The advantage of a narrow range in genuine goods is obvious, and were this the only consideration one need have no hesitation in recommending the adoption of the 1.0 cc. method. However, the lead value of the tentative method had a peculiar merit in that upon adulteration of maple sirup with refined sugar sirup it fell off not simply proportionally to the true maple content but at a more rapid rate, such that the lead value reached zero when the maple content was as high as 20 per cent. Analyst A has

made some experiments to determine whether this advantage inheres also in the modified methods. Results on three sirups with lead values of about 4 (3.65, 4.42 and 4.12 by the tentative method) indicate that with the use of 1.0 cc. of reagent much of this advantage is sacrificed. The Fowler method, however, as might be expected, gives results similar in this respect to the tentative method.

TABLE 7.

Lead numbers of adulterated samples.

NUMBER	B	A	K	T	S	H	R	F	MEAN	C
					Tentative Method:					
2	4.29	3.95	3.95	4.02	3.71	3.17	3.32		3.77	4.93
3	1.48	1.62	1.47	2.06	1.47	1.40	1.52		1.57	2.52
5	1.89	1.98	1.71	2.21	1.47	1.73	1.56		1.79	2.02
6	1.25	1.26	1.15	1.87	0.84	1.09	1.03		1.21	1.50
29	2 62	2.82	2.81	3 41				2 84	2.90	
30	1.20	1.23	1.16	1.89				1 27	1.35	
					Fowler Method:					
2	3.74	4.05	5.11		4.14	3.47	3.47		4.00	4.92
3	1.63	1.82	1.95		1.80	1.61	1.60		1.74	2.97
5	1.90	2.20	2.30		1.74	1.87	1.76		1.96	2.46
6	1.30	1.38	1.59		1.14	1.17	1.18		1.29	1.65
29	2 66	2.98	3.17					3.03	2.96	
30	1.28	1.41	1.53					1 45	1.42	
					Proposed Method (1 cc.):					
2	3.64	3.91	4 59	3 75	4.15	3.29	3.13		3.78	4.88
3	1 86	2.10	2.26	2.22	2.10	1.84	1.87		2.04	2.74
5	2.25	2.46	2.61	2.33	2.13	1.95	2.04		2.25	2.80
6	1.74	1 74	1.92	1.99	1.58	1.40	1.54		1.70	1.66
29	2.64	3.06	3.39	2.98				3 18	3.05	
30	1.59	1.77	1 79	2.04				1.69	1.78	

Table 7 gives the mean lead values found by each analyst in the six adulterated sirups and Table 8 a comparison of the mean results of all analysts on these with those on the pure sirups from which they were derived. The range of individual analysts' results on the 47 pure sirups to which the three modifications of the Canadian lead value have been applied (see above) is as follows: Tentative method 2.02–6.16; Fowler meth-

TABLE 8.

Comparison of lead values in pure sirups and their adulterated derivatives.

(Mean results of all analysts)

PURE SIRUPS				20% ADULTERATED				GROSSLY ADULTERATED			
No.	Tentative	Fowler	1 cc.	No	Tentative	Fowler	1 cc.	No.	Tentative	Fowler	1 cc.
1	4.77	4.86	4.21	2	3.77	4.00	3.78	3	1.57	1.74	2.04
4	2.43	2.72	2.87	5	1.79	1.96	2.25	6	1.21	1.29	1.70
28	3.64	3.84	3.72	29	2.90	2.96	3.05	30	1.35	1.42	1.78

TABLE 9.

Variation of duplicates.

(Average differences between the maximum and minimum results of each analyst on each sirup)

	B	A	K	T	S	H	R	F	Av.	C
Sirups analyzed	30	30	30	30	20	15	9	10		20
Tentative method	0.040	0.080	0.102	0.082	0.085	0.115	0.033	0.045	0.076	0.13
Fowler method	0.039	0.070	0.090	—	0.043	0.063	0.067	0.053	0.062	0.25
1 cc. method	0.042	0.079	0.064	0.087	0.058	0.070	0.050	0.045	0.063	0.24

TABLE 10.
Conductivity values (pure samples)

NO.	22 GRAMS DRY MATTER									25 GRAMS DRY MATTER								
	M	A	K	S	H	F	Mean	Deviation from mean +	Deviation from mean −	M	A	K	S	H	F	Mean	Deviation from mean +	Deviation from mean −
1	180	178	179	174	177		177.3	2.7	3.3	184	179	180	178	182		180.6	3.4	2.6
4	132	129	132	132	133		131.6	1.4	2.6	136	129	133	135	135		133.6	2.4	4.6
7	150	143	161	141	148		148.6	12.4	7.6	153	144	162	147	144		150.0	12.0	6.0
8	165	154	162	145	159		156.8	8.2	11.8	168	153	161	149	165		159.2	8.8	10.2
9	162	156	164	139	159		156.0	8.0	17.0	165	156	161	142	166		158.0	8.0	16.0
10	160	156	180	154	162		162.4	17.6	8.4	164	158	184	156	167		165.8	18.2	9.8
11	161	156	174	158	161		162.0	12.0	6.0	165	156	170	160	162		162.6	7.4	6.6
12	162	154	173	144	167		160.0	13.0	16.0	165	156	169	144	173		161.4	11.6	17.4
13	151	146	169	144	154		152.8	16.2	8.8	154	144	165	151	155		153.8	11.2	9.8
14	141	135	155	138	140		141.8	13.2	6.8	145	138	152	133	151		143.8	8.2	10.8
15	146	144	160	133	150		146.6	13.4	13.6	149	146	158	139	156		149.6	8.4	10.6
16	161	154	179	154			162.0	17.0	8.0	164	154	175	156			162.3	12.7	8.3
17	140	—	154	127			140.3	13.7	13.3	143	132	147	127			137.3	9.7	10.3
18	136	131	150	128			136.3	13.7	8.3	140	132	149	127			137.0	12.0	10.0
19	184	178	210	175			186.8	23.2	11.8	189	179	205	183			189.0	16.0	10.0
20	174	169	193	164			175.0	18.0	11.0	179	173	189	175			179.0	10.0	6.0
21	171	164	192			175	175.4	16.6	11.4	176	173	189			171	176.8	12.2	5.8
22	177	176	202			172	181.8	20.2	9.8	182	178	197			176	183.3	13.7	7.3
23	171	—	168			162	167.0	4.0	5.0	175	167	164			168	168.5	6.5	4.5
24	166	160	171			158	163.8	7.2	5.8	170	162	173			162	166.8	6.2	4.8
25	165	160	186			160	167.8	18.2	7.8	170	166	178			162	169.0	9.0	7.0
26	140	126	144			130	135.0	9.0	9.0	137	126	139			132	133.5	5.5	7.5
27	134	127	148			129	134.5	13.5	7.5	137	131	143			131	135.5	7.5	4.5
28	157	149	166			153	156.3	9.7	7.3	161	149	161			155	156.5	4.5	7.5
	Average						157.4	12.6	9.1							158.9	9.4	8.2
	Maximum						186.8	on No. 19								189.0	on No. 19	
	Minimum						131.6	on No. 4								133.5	on No. 26	
	Difference						55.2									55.5		
	Range % of Minimum						41.9									41.6		
	Range % of Average						35.1									34.9		

TABLE 11.

Conductivity values (adulterated samples)

NO.	22 GRAMS DRY MATTER							25 GRAMS DRY MATTER						
	M	A	K	S	H	F	Mean	M	A	K	S	H	F	Mean
2	148	—	132*	145	148		147.0	152	146	153	145	155		150.2
3	94	94	69*	94	94		94.0	95	94	96	94	99		95.6
5	109	100	104	105	107		105.0	112	105	111	102	105		107.0
6	89	88	91	89	88		89.0	91	92	87	88	91		89.8
29	131	126	142	125		125	131.0	134	126	137			126	130.8
30	83	80	98	78		78	84.8	84	82	93			79	84.5

* Designated doubtful by the analyst. Omitted in averaging.

od 2.32–6.37; 1 cc. method 2.28–5.47. In all three methods the highly adulterated samples give lead values below the minima found in these pure sirups. Of the moderately adulterated ones only that (No. 5) made from a pure sirup of comparatively low lead value gives a lower value than the minimum found in genuine sirups. In that sirup the means of the values found by all analysts by the tentative and Fowler methods are decidedly, but that found by the 1 cc. method only slightly, below the minimum reported by any analyst on the 47 genuine sirups. In some other instances results on the moderately adulterated samples are lower than the minimum found *by the same analyst* in the genuine sirups of this year's collection.

Table 9 compares the variability of duplicate determinations by the three methods in the hands of each analyst. All the samples, pure and adulterated, are included. Although one analyst (R), working on 9 samples, obtained closer duplicates by the hot washing method and four others (B, A, T and F) got very good and nearly equal agreement by all three methods, the others (K, S and H) had decidedly better success with the cold washing methods than with the tentative method, and the average is in favor of those methods.

Evidently the merits of the 1.0 cc. and 1.5 cc. methods deserve further study. Meanwhile, considering that the Fowler method possesses advantages over the tentative one in respect to range of genuine values, and to agreement of duplicates and its adoption would involve no radical change in the judgment of samples, there would appear to be no objection to substituting that method for the present tentative one.

CONDUCTIVITY VALUES

The instructions issued for collaborative work on the conductivity value were to make the determinations according to the tentative method (to which a slight editorial modification was adopted last year) and also to make determinations according to the same method, using the quantity of sirup containing 25 grams instead of 22 grams of dry matter. The results reported in Tables 10 and 11 show that, almost invariably, slightly higher results are obtained when the quantity of dry matter taken is 25 grams. The range of values in the 24 pure sirups is practically the same by both methods. Accordingly there appears no reason why the use of 25 grams of dry matter should not be substituted for that of 22 grams. This would make it possible to use the same dilution for Canadian lead value and conductivity value.

As a rule, though not without exceptions, Analyst K obtained higher conductivity values than any of the other collaborators. On the 22 gram method his results on Sirups Nos. 19 and 22, viz. 210 and 202, and in the 25 gram method his result on No. 19, viz. 205 (which is lower than where

22 grams was used), were the highest conductivity values reported on genuine sirups. The lowest conductivity value found on this year's sirups was 126—by A on No. 26 by both methods.

The grossly adulterated samples gave conductivity values below the minimum reported up to the present on a genuine sirup. The moderate adulterations would escape definite detection by this method, although doubt would be cast upon the purity of one of the samples (No. 5).

For recommendations regarding future work and those pertaining to the revision of *Methods of Analysis*, see *This Journal*, **14**, 43, 76 (1931).

No report on starch conversion products was given by the associate referee.

No report on drying, densimetric and refractometric methods was given by the associate referee.

REPORT ON POLARISCOPIC METHODS

By F. W. ZERBAN (New York Sugar Trade Laboratory, New York, N. Y.), *Associate Referee*

I. EFFECT OF HYDROCHLORIC ACID AT VARIOUS TEMPERATURES ON THE ROTATION OF DEXTROSE AND LEVULOSE IN THE PRESENCE OF AMINO COMPOUNDS

In the report of the associate referee for 1929[1] it was pointed out that both the official invertase method and Jackson and Gillis method No. II should yield zero sucrose in the analysis of invert sugar, even in the presence of amino compounds. It was actually observed, however, that although the invertase method gave the theoretical result in the case of pure invert sugar, small percentages of apparent negative sucrose were indicated by the analysis of its mixtures with asparagine or aspartic acid. Similarly, Jackson and Gillis method No. II gave slightly negative sucrose figures, both with invert sugar itself and in the simultaneous presence of amino compounds. It was also found that in most of the cases cited the analytical results obtained in the two laboratories where the work was carried out did not check very closely.

To explain the apparent negative sucrose result shown by Jackson and Gillis method No. II in the analysis of pure invert sugar, it was suggested that even at the comparatively low temperature of 26°–27°C. the levulose may not be entirely stable in the presence of hydrochloric acid. The low results and poor checks obtained with both the invertase method and

[1] *This Journal*, **13**, 188 (1930).

Jackson and Gillis method No. II in the analysis of mixtures of invert sugar with amino compounds were tentatively explained by a reaction of the amino compounds with the reducing sugars. But it was necessary to test these hypotheses experimentally, and for this reason it was recommended that a fundamental study be made of the various factors which may affect the determination of sucrose in the presence of invert sugar and of amino compounds.

This investigation was undertaken in the New York Sugar Trade Laboratory, the experimental work being carried out by C. A. Gamble and J. E. Mull. The writer is indebted also to C. F. Snyder, for a supply of Bureau of Standards dextrose, and to R. F. Jackson for the pure levulose furnished by him.

The following materials were used in this investigation, each diluted with water to a total volume of 100 ml.:

(1) 6.5 grams dextrose (levulose).

(2) 6.5 grams dextrose (levulose), plus 10 ml. of a solution containing 0.26 gram l-asparagine neutralized to *p*H 7 with sodium hydroxide.

(3) 6.5 grams dextrose (levulose) plus 10 ml. of a solution containing 0.26 gram l-aspartic acid neutralized to *p*H 7 with sodium hydroxide.

(4) 6.5 grams dextrose (levulose) plus 10 ml. hydrochloric acid of sp. gr. 20:4 = 1.1029.

(5) Same as under (4), but with the further addition of asparagine solution as specified under (2).

(6) Same as under (4), but with the further addition of aspartic acid solution as specified under (3).

The flasks were then filled with water nearly to the mark, and allowed to stand for 24 hours. In one series of experiments the six flasks were kept in a refrigerator at a temperature of 8°C., and in the other four series in an incubator at 20°, 30°, 40°, and 50°C., respectively. At the expiration of the 24 hour period the temperature of all flasks was adjusted to 20°C., and the volume was completed to 100 ml. Ten readings were taken in the saccharimeter at exactly 20°C. in a 200 mm. tube, and the readings were averaged. The entire experimental work was carried out independently by two analysts. The results are shown in Tables 1 and 2.

Dextrose in pure aqueous solution is shown to be quite stable even at 50°C. Levulose suffers slight decomposition at that temperature, but not at 40° or below.

In the presence of hydrochloric acid only, dextrose is not attacked even at 50°C., but levulose undergoes partial destruction at 40°, to a more pronounced degree at 50°, and incipient decomposition is noticeable even at 30°.

When asparagine or aspartic acid is added to dextrose, but no hydrochloric acid, decomposition begins to show at 50°, but not at 40°. In the case of levulose, decomposition is marked at 30°, becomes more pro-

nounced at 40°, and is strong at 50°. But when hydrochloric acid is added, besides asparagine or aspartic acid, dextrose is quite stable at as high a temperature as 50°, and even levulose does not decompose at 30°, although at 40° and 50° it is attacked to a considerable extent.

TABLE 1.

Dextrose.

ANALYST	TEMPERATURE	DEXTROSE ALONE	DEXTROSE + ASPARAGINE	DEXTROSE + ASPARTIC ACID	DEXTROSE + HCl	DEXTROSE + ASPARAGINE + HCl	DEXTROSE + ASPARTIC ACID + HCl
	°C.	°V.	°V.	°V.	°V.	°V.	°V.
C. A. Gamble	8	19.80	19.60	19.50	19.90	20.22	20.19
J. E. Mull		19.80	19.56	19.49	19.89	20.29	20.23
Average		19.80	19.58	19.49	19.90	20.26	20.21
C. A. Gamble	20	19.80	19.59	19.51	19.88	20.20	20.17
J. E. Mull		19.76	19.57	19.50	19.86	20.26	20.19
Average		19.78	19.58	19.51	19.87	20.23	20.18
C. A. Gamble	30	19.83	19.56	19.50	19.85	20.20	20.15
J. E. Mull		19.86	19.60	19.56	19.86	20.22	20.18
Average		19.84	19.58	19.53	19.86	20.21	20.16
C. A. Gamble	40	19.80	19.56	19.52	19.85	20.20	20.17
J. E. Mull		19.85	19.57	19.52	19.91	20.26	20.22
Average		19.83	19.56	19.52	19.88	20.23	20.20
C. A. Gamble	50	19.83	19.34	19.28	19.86	20.20	20.15
J. E. Mull		19.80	19.37	19.31	19.87	20.16	20.18
Average		19.82	19.35	19.30	19.86	20.18	20.17

The net result, from the standpoint of the present inquiry, is that in the neighborhood of 30° levulose is slightly attacked by hydrochloric acid in the absence of amino compounds, and by amino compounds in the absence of hydrochloric acid, but not when both are present simultaneously. Dextrose is much more stable under all conditions, and only shows slight decomposition at 50° in the presence of amino compounds alone, without hydrochloric acid.

The apparent negative sucrose found last year in the analyses of mixtures of invert sugar with amino compounds by the invertase method can not therefore be explained by an interaction of the constituents at the temperature used. It appears that the explanation offered in the report of the associate referee for 1928 is the correct one after all. In the tests reported in 1928 and 1929 there was a difference in *p*H between the solutions used for the direct and the invert reading, owing to the addition of acetic acid in the latter case. With invert sugar alone, the effect of the acetic acid on the rotation is negligible, and for this reason the analyses gave

correct results of practically zero sucrose. But in the presence of asparagine or aspartic acid a difference in *p*H produces a pronounced effect as previously shown, particularly by Liquier.[1]

According to Table 1, the hydrochloric acid used raises the positive ro-

TABLE 2.

Levulose.

ANALYST	TEMPERATURE	LEVULOSE ALONE	LEVULOSE + ASPARAGINE	LEVULOSE + ASPARTIC ACID	LEVULOSE + HCl	LEVULOSE + ASPARAGINE + HCl	LEVULOSE + ASPARTIC ACID + HCl
	°C	°V.	°V.	°V.	°V	°V.	°V.
C. A. Gamble	8	−34.60	−34.71	−34.81	−35.27	−34.92	−35.00
J. E. Mull		−34.64	−34.75	−34.80	−35.23	−34.87	−34.95
Average		−34.62	−34.73	−34.80	−35.25	−34.89	−34.97
C. A. Gamble	20	−34.66	−34.77	−34.80	−35.21	−34.90	−34.94
J. E. Mull		−34.65	−34 72	−34.79	−35.29	−34.81	−34.98
Average		−34.66	−34.74	−34.79	−35.25	−34.85	−34.96
C. A. Gamble	30	−34.70	−34.70	−34.70	−35.21	−34.89	−35.00
J. E. Mull		−34.61	−34 61	−34.62	−35.18	−34.83	−34.94
Average		−34.65	−34.66	−34.66	−35.19	−34.86	−34.97
C. A. Gamble	40	−34.72	−34 30	−33.98	−34.81	−34.60	−34.76
J. E. Mull		−34.66	−34.33	−34.05	−34.86	−34.66	−34.50
Average		−34.69	−34.32	−34.02	−34.83	−34.63	−34.63
C. A. Gamble	50	−34.50	−33.05	−32.28	−33.40	−33.40	−33.82
J. E. Mull		−34.56	−33.65	−32.76	−34.08	−33.90	−34.05
Average		−34.53	−33.35	−32.52	−33 74	−33.65	−33.94

tation of the quarter normal weight of dextrose plus asparagine or aspartic acid by 0.7°, but increases the negative rotation of the quarter normal weight of levulose, under the same conditions, by only 0.16°. Therefore, in the presence of asparagine or aspartic acid, hydrochloric acid shifts the rotation of invert sugar to the right, or in other words, decreases numerically the negative rotation. Thus the increase in actual acidity of the solution used for the invert reading tends to give a negative result for sucrose. It may be concluded that when working with invertase more accurate results may be obtained if the reaction of the solutions used for the direct and invert readings is made exactly the same immediately before the readings are taken.

Turning now to Jackson and Gillis method No. II, the small quantity of apparent negative sucrose found in the analyses of pure invert sugar, without amino compounds, is reasonably attributable at least in part to

[1] *Comp. rend.*, **180**, 1917 (1925).

a slight decomposition of the levulose by the effect of the hydrochloric acid at 26°–27°C., as suggested last year.

But it is evident from the tables that the apparent negative sucrose found for the mixtures containing also amino compounds cannot be ascribed to an interaction of the latter with the invert sugar at 26°–27°C. because when hydrochloric acid is present even levulose is quite stable at that temperature. On the contrary, it might more reasonably be expected to find traces of apparent sucrose, because in the absence of hydrochloric acid levulose is slightly attacked by amino compounds at 30°.

The finding of apparent negative sucrose in these mixtures must therefore be explained in some other way. Throughout this entire investigation, begun in 1924, it has been repeatedly noticed that Jackson and Gillis method No. II showed a general tendency toward low results for sucrose. The report for 1924 commented on this point as follows: "In a number of determinations made in this laboratory, it has been found that it is very difficult to obtain good checks in the invert readings with this method. A great deal seems to depend on the exact way in which the neutralization with ammonia is carried out." The addition of ammonia to the strongly acid solution not only raises the temperature of the solution considerably, but there is also a chance of a local effect of the ammonia, not yet neutralized, on the invert sugar, with a consequent attack on the latter.

To test this point still further, the following experiments were carried out. Quarter normal solutions of dextrose and of levulose were prepared, some without amino compounds, and others with the addition of either asparagine or aspartic acid in the same quantities as used in the tests shown in Tables 1 and 2. Then, in parallel experiments, hydrochloric acid was first added and this immediately neutralized with ammonia in one case, while in the other the equivalent quantity of ammonium chloride was added directly, just as is done in Jackson and Gillis method No. II. It was found that with dextrose, either alone or in the presence of amino compounds, the two parallel tests gave the same results within 0.05°V., whether the ammonium chloride was added directly or formed in the solution from its components. Levulose alone also showed no difference. But in the mixtures of levulose with aspartic acid or asparagine the rotation of the neutralized solution was from 0.1° to 0.2° lower than when the ammonium chloride was added as such.

The explanation for the finding of apparent negative sucrose by Jackson and Gillis method No. II must therefore be sought partly in the execution of the method itself, and partly in the slight destructive effect of hydrochloric acid on the levulose during a period of 24 hours at 26°–27°C., as mentioned above. In carrying out the neutralization with ammonia in Jackson and Gillis method No. II, it is obviously necessary to add the ammonia very slowly under constant agitation, at the same time avoiding a temperature rise, by artificial cooling if necessary.

II. EFFECT OF CLARIFICATION WITH LEAD SUBACETATE SOLUTION ON THE DETERMINATION OF SUCROSE BY INVERSION WITH INVERTASE

Much time and labor has been devoted by numerous investigators to studies concerning the effect of lead subacetate and other clarifying agents on the rotation of sucrose, reducing sugars, and non-sugars, and on the direct polarization of sugar products, but little information is available regarding the effect of clarification on sucrose determination by Clerget analysis. Jackson and Gillis[1] have presented a table showing in what way clarification with lead subacetate affects separately the sucrose, the invert sugar originally present and the "non-sugars of the beet." Some work has also been done with the direct object of evaluating the influence of clarification with lead subacetate on the determination of sucrose by acid hydrolysis. Cross[2] has contributed to this subject and has reviewed earlier literature on it. But in all these investigations the only basis of comparison by which the accuracy of the results could be judged was the particular "standard" method in vogue at the time.

The results obtained by the associate referee during the past six years have conclusively demonstrated that in the analysis of complex mixtures like cane products, the invertase method is the only safe procedure to be followed for the determination of sucrose. All this work was done with mixtures or products that required no clarification. But in practice clarification is almost always a necessary preliminary step and its effect on the determination of sucrose must be accurately known before it can be definitely stated that a sucrose result obtained actually represents the quantity of sucrose in a product, according to the present state of our knowledge.

The most direct way to test this point is to analyze a natural or manufactured product which is light enough in color to require no clarification, and to make parallel analyses after use of the various clarifying agents commonly employed. Preferably a low purity product should be chosen because this would accentuate the effect of non-sucrose constituents.

Unfortunately it is not an easy matter to find products of this description. Cane molasses is always much too dark to be read except in excessive dilution, and even a cane sirup of sufficiently light color is probably a rare exception. It has therefore been necessary to turn to refinery products as a first step. Several refineries kindly submitted samples of barrel sirups, and among these two were found which at a concentration of the half-normal weight in 100 ml. could be read directly in the saccharimeter.

Bone black treated products are admittedly different in composition from the products of the raw sugar factory, especially as regards the ratio between dextrose and levulose. Refinery sirups usually contain less levu-

[1] Bur. Standards Sci. Paper 375, p. 180 (1920).
[2] Louisiana Bull. 135, p. 25 (1912).

lose than dextrose, while in normal cane products these two sugars are about equal in quantity. It should be pointed out, therefore, that any results obtained with refinery sirups are not directly applicable to raw sugar products.

The experimental work was carried out by S. Byall, Bureau of Chemistry and Soils, and C. A. Gamble and J. E. Mull, New York Sugar Trade Laboratory. To all of these the writer wishes to express his sincere thanks.

Some preliminary tests showed that the half-normal weight of sirup A (Table 3) required about 8 ml. of lead subacetate solution for the complete precipitation of the non-sugars removable in this way, and Sirup B about 12 ml. The quantities of lead subacetate solution used in the experiments were chosen so that an insufficient amount as well as an increasing excess was used.

The complete work plan was as follows:

(1) *Polarization and sucrose without lead clarification*

Dissolve 52 grams of sirup in water to 200 cc. total volume.

Direct polarization.—Pipet 50 cc. of this solution into a 100 cc. flask, make up to volume, mix well, and let stand in a stoppered flask to complete mutarotation. Take a reading at 20° in a 200 mm. tube, and multiply result by 2. Report this figure for each sirup.

Invert polarization.—Pipet another 50 cc. portion of the original solution (52 grams of sirup to 200 cc.) into a 100 cc. flask, acidify with acetic acid to about *p*H 4.6, add 10 cc. of invertase (k = 0.1), and about 20 cc. of water. Mix well and let stand overnight to complete inversion. Then make up to mark, mix again, and let stand in a stoppered flask to complete mutarotation. Take reading and report figure as above for each sirup.

(2) *Polarization and sucrose with lead clarification*

Transfer four portions of sirup, of 65 grams each, to four 250 cc. flasks and dissolve in a small quantity of water. Add the following quantities of lead subacetate solution of 1.25 sp. gr. (*Methods of Analysis, A.O.A.C.*, p. 182, **18(a)**:

Sample from Refinery (A): (a) 20 cc.; (b) 40 cc.; (c) 60 cc.; (d) 80 cc., respectively.

Sample from Refinery (B): (a) 30 cc.; (b) 60 cc.; (c) 90 cc.; (d) 120 cc. respectively.

After adding the lead, mix well, make up to mark, shake and filter, discarding the first runnings.

Direct polarization without deleading.—Pipet 50 cc. of filtrate from the lead precipitate into a 100 cc. flask, make up to volume, mix well, and let stand in a stoppered flask to complete mutarotation. Take readings and report as above for each of the four quantities of lead added and for each sirup.

Direct polarization after deleading (P).—Delead the remainder of the filtrate from the lead precipitate with solid ammonium dihydrogen phosphate, and filter, discarding the first runnings. Pipet 50 cc. of the deleaded solution into a 100 cc. flask, make up to volume, mix well, and let stand in a stoppered flask to complete mutarotation. Take readings and report as above for each of the four quantities of lead added and for each sirup.

Invert polarization after deleading (I).—Pipet another 50 cc. portion of the deleaded solution into a 100 cc. flask, and add 10 cc. of invertase (k = 0.1) and about

20 cc. of water. Mix well and let stand overnight to complete inversion. Make up to volume, mix again, and let stand in a stoppered flask to complete mutarotation. Take readings as above, and report for each of the four quantities of lead added and for each sirup.

All the work is to be done in duplicate, preferably by two different chemists.

The results of the analyses are assembled in Table 3. The quantities of lead subacetate solution shown are on the basis of 65 grams, or five half-normal weights.

In some cases the readings obtained in the two laboratories check closely, while in others there are rather large discrepancies. This may be due, at least partly, to the fact that in the New York Sugar Trade Laboratory the work was done in the late winter directly after receipt of the samples, while in Washington the analyses were not made until midsummer. The composition of the sirups may have changed somewhat during the interval. However, since in either laboratory the analyses were made at one time, the averages may be used as a basis of comparison.

Considering first the sum of the direct and invert readings after deleading, which is the most important criterion, it is found that in Sirup A the entire range, from no clarification to the largest excess of lead, is from 22.90 to 23.08, with an average of 23.00. The changes with increase of lead are in both directions. It may be concluded that in the case of this sirup even a large excess of lead does not materially affect the sucrose result, provided the excess lead is removed before both the direct and invert readings are taken.

The direct polarization, before deleading, shows a slight decrease upon the addition of the smallest quantity of lead used, and then rises slowly with increasing quantities of lead. This is quite in line with previous observations.

In the case of Sirup B there is a difference of about 1 per cent between the sucrose figures found in the two laboratories, without clarification. The results with lead clarification show much better agreement. The average sums of the direct and invert readings after deleading vary only from 28.51 to 28.63, again moving up and down irregularly. The average for the unclarified sirup is 28.61, and if this be accepted, the general results agree quite well with those obtained in the analyses of Sirup A. In other words, the curve showing the relation between $(P+I)$ and ml. of lead solution used is practically a straight line. If this line be extrapolated to zero lead for Sirup B, the value 28.57 is found.

The direct polarization before deleading rises gradually from no lead to the largest quantity of lead added.

No definite conclusions can be drawn from these two series of experiments, but the indications are that even a large excess of lead subacetate does not materially affect the sucrose results obtained for refinery sirups by inversion with invertase, provided the excess lead is removed from the

TABLE 3.

Sirup A.

QUANTITY OF LEAD, AND ANALYST	DIRECT POLARIZATION BEFORE DELEADING	DIRECT POLARIZATION AFTER DELEADING (*P*)	INVERT POLARIZATION AFTER DELEADING (*I*)	*P+I*	SUCROSE (PER CENT)
No lead, B.	17.72	17.72	−5.25	22.97	34.78
No lead, G. & M.	17.50	17.50	−5.50	23.00	34.82
Average	17.61	17.61	−5.38	22.99	34.80
20 ml., B.	17.41	17.28	−5.70	22.98	34.79
20 ml., G. & M.	17.48	17.43	−5.65	23.08	34.94
Average	17.45	17.36	−5.68	23.03	34.87
40 ml., B.	17.36	17.23	−5.66	22.89	34.66
40 ml., G. & M.	17.53	17.43	−5.68	23.11	34.99
Average	17.45	17.33	−5.67	23.00	34.83
60 ml., B.	17.69	17.25	−5.54	22.79	34.50
60 ml., G. & M.	17.60	17.45	−5.55	23.00	34.82
Average	17.65	17.35	−5.55	22.90	34.66
80 ml., B.	17.68	17.38	−5.62	23.00	34.82
80 ml., G. & M.	17.85	17.63	−5.53	23.16	35.06
Average	17.77	17.51	−5.58	23.08	34.94
		Sirup B.			
No lead, B.	20.97	20.97	−7.96	28.93	43.80
No lead, G. & M.	20.58	20.58	−7.71	28.29	42.82
Average	20.78	20.78	−7.84	28.61	43.31
30 ml., B.	21.25	20.94	−7.59	28.53	43.20
30 ml., G. & M.	20.80	20.80	−7.68	28.48	43.12
Average	21.03	20.87	−7.64	28.51	43.16
60 ml., B.	21.69	21.42	−7.19	28.61	43.32
60 ml., G. & M.	21.30	21.13	−7.38	28.51	43.16
Average	21.50	21.28	−7.29	28.56	43.24
90 ml., B.	21.95	21.61	−6.99	28.60	43.30
90 ml., G. & M.	21.80	21.65	−7.00	28.65	43.38
Average	21.88	21.63	−7.00	28.63	43.34
120 ml., B.	22.77	21.69	−6.79	29.48	43.12
120 ml., G. & M.	22.35	21.15	−7.40	28.55	43.23
Average	22.56	21.42	−7.10	28.52	43.18

solution before the direct and invert readings are taken. If these results should be confirmed by further work, it is evident that the volume error caused by the precipitate must be compensated by some other error. It should be pointed out again that entirely different results may be obtained with sirups and molasses produced in the raw sugar factory

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of the effect of lead clarification on the determination of sucrose in cane products be continued and extended to products of the raw sugar factory.

(2) That the study of inversion procedures for beet products, interrupted some years ago, be taken up again, with special reference to those containing raffinose.

REPORT ON CHEMICAL METHODS FOR REDUCING SUGARS

By R. F. JACKSON (Bureau of Standards, Washington, D. C.),
Associate Referee

The reducing sugar methods that have previously been adopted by this association require for their most efficient operation relatively large and concentrated samples of reducing sugar. A useful addition to the list of methods would be one which could be accurately carried out with small samples. Such a method is the one described by Scales,[2] which is at its best at the concentrations of sugar in which the adopted methods are conducted with greatest uncertainty. The associate referee and his collaborators have conducted preliminary experiments, and while they are unable to obtain results of the precision claimed by the author of the method, they have found that samples containing from 10 to 20 mg. of sugar can be analyzed with an accuracy of about 1 per cent. Further work on the method is contemplated. It is recommended that the method be adopted as tentative.[1]

COMMITTEES NAMED BY THE PRESIDENT

Committee to Wait Upon Secretary of Agriculture: C. C. McDonnell, J. A. Le Clerc and W. C. Geagley.

Committee on Resolutions: L. D. Haigh and C. A. Browne.

Committee on Auditing: A. S. Mitchell, G. G. Frary and L. H. Bailey.

Committee on Nominations: W. H. MacIntire, R. N. Brackett and C. S. Cathcart.

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 45 (1931).
[2] *Ind. Eng. Chem.*, 11, 747 (1919).

FIRST DAY

MONDAY—AFTERNOON SESSION

REPORT ON FERTILIZERS

By G. S. FRAPS (Agricultural Experiment Station, College Station, Tex.), *Referee*

The progress made by the associate referees will be presented in their reports. The most important matter to come up relates to the estimation of available phosphoric acid in fertilizers. Superphosphate will absorb approximately 2 per cent ammonia, without change in availability. If larger amounts of ammonia are added, there is a decrease in available phosphoric acid as measured by the present methods. Comparatively slight modifications in the quantity of fertilizer and the time of digestion increase the apparent availability of the phosphoric acid. A modification of the method would encourage the use of liquid ammonia in preparing fertilizers and probably help to decrease the cost of nitrogen in fertilizers. Questions to be decided are the compounds formed when the phosphoric acid is made insoluble (as measured by the present methods) and the availability to plants of this phosphoric acid.

This matter has been given careful, thorough and extensive study by the Associate Referee on Phosphoric acid, W. H. Ross, and the results will be presented in full in his report. This is an important matter, and it should receive careful consideration.

The Associate Referee on Nitrogen Activity Methods in Fertilizers recommends that the changes approved last year (with a correction) be finally adopted as official. The referee concurs in this recommendation and also in the recommendations of the other associate referees.

Since this work is now satisfactorily completed, the referee recommends that the work on nitrogen activity in fertilizers be discontinued.

REPORT ON PHOSPHORIC ACID

AVAILABILITY OF THE REVERTED PHOSPHORIC ACID IN AMMONIATED SUPERPHOSPHATES

By WM. H. ROSS, *Associate Referee*, and K. D. JACOB (Fertilizer and Fixed Nitrogen Investigations, Bureau of Chemistry and Soils, Washington, D. C.)

Phosphatic fertilizer materials may be classified according to their usage into two groups: (1) Those that are usually applied to the soil separately, such as raw bone and ground phosphate rock, and (2) those that are also

used in fertilizer mixtures, such as superphosphate and ammonium phosphate.

Certain materials of the first group have been used as fertilizers since earliest times. Those of the second group are usually prepared by chemical treatment of the materials of the first group, and their manufacture, which began in England in 1842, marks the beginning of the commercial fertilizer industry.

Although the chemical treatment to which the first group materials are subjected in the manufacture of commercial fertilizers increases their availability, it does not follow that a quickly available phosphate is always more desirable than one that is less readily available. The phosphoric acid in steamed bones is more slowly available than that in superphosphate, but the former commands a higher price per unit of P_2O_5 than the latter, owing to the fact that it can be used with greater safety on certain types of plants and for other reasons.

There is a demand, however, for materials containing phosphoric acid that can be readily utilized by the first crop following its application, and considerable attention was given in the early history of the industry to the development of a laboratory method that would distinguish between such materials and those that are less readily available. It was observed that certain forms of water-insoluble phosphoric acid were just as available as those that are more readily soluble and that water solubility, as applied in the analysis of potash salts, could not be used as a true measure of phosphoric acid availability.

The use of ammonium citrate solution as a means of measuring the availability of phosphates was first proposed by Fresenius, Neubauer and Luck[1] in 1871. This reagent had long been used in the determination of total phosphoric acid by a method that is still used officially in Germany and other European countries. The method consists in treating a solution of the sample to be analyzed with ammoniacal ammonium citrate solution and then adding magnesia mixture to precipitate the phosphoric acid present. The situation of having a citrate method for determining total phosphoric acid and a citrate method for determining the availability of phosphates makes a search of the literature on either method a confusing one.

A modification of the method of Fresenius, Neubauer and Luck was adopted[2] by this association at its first meeting in 1884, and this method with minor changes still remains the official method for measuring the availability of the phosphoric acid in superphosphates and fertilizer mixtures in general.

Although a neutral solution of ammonium citrate had thus been adopted as a means of measuring the availability of superphosphates it was

[1] *Z. anal. Chem.*, **10**, 149 (1871).
[2] *Proc. Assoc. Official Agr. Chem.*, 1884, p. 4.

claimed to be of little value when applied to basic slags. In 1894 Wagner[1] proposed the use of an acid ammonium citrate solution for evaluating this material, but Gerlach and Passon[2] later reported that the active agent in the ammonium citrate solution was the free citric acid that it contained and that the use of ammonia in the preparation of the solution might be omitted. Wagner[3] approved this change in the method after many vegetative tests and finally decided on a 2 per cent citric acid solution as the most satisfactory to use. This method was adopted tentatively[4] by this association in 1911, and became the official method[5] for measuring the availability of basic slags in 1922.

A few years ago a third water-insoluble material, known as precipitated phosphate or dicalcium phosphate, came on the fertilizer market. It was shown by Haskins[6] that the availability of this material was greater than that indicated by the neutral ammonium citrate test, which specifies the digestion of 2 grams of sample in 100 cc. of solution, but that the method could be made applicable to the analysis of this material by the simple modification of reducing the weight of the sample from 2 grams to 1 gram. This change, which represents the second that has been made in the official method as new materials came on the market, was adopted[7] by the association in 1922.

Within the past two or three years commercial methods have been developed for the direct utilization of free ammonia in fertilizers by absorption in superphosphates. This treatment tends to reduce the water-soluble and increase the citrate-insoluble phosphoric acid in the superphosphate. Little change occurs in the amount of citrate-insoluble phosphoric acid up to an absorption of about 2 per cent of ammonia, but this amount gradually increases with increase in ammonia content up to the maximum absorption under present commercial practice of about 6 per cent when the citrate-insoluble phosphoric acid may approach or even exceed 6 per cent.

That a superphosphate treated in this way behaves differently from the ordinary superphosphate was first called to the attention of the Associate Referee on Phosphoric Acid by R. M. Jones, a former employee of the Bureau of Chemistry and Soils, who found that the citrate-insoluble phosphoric acid in ammoniated superphosphates increased as the water-insoluble residue was allowed to stand before digestion in the ammonium citrate solution. This was later confirmed by Howes and Jacobs[8] of the Davison Chemical Company, who also found that the citrate-insoluble phosphoric acid in ammoniated superphosphates varied greatly with (1) the weight of the sample taken for analysis; (2) the acidity of the solution;

[1] Düngungsfragen, Berlin, 1894.
[2] *Chem. Ztg.*, 20, 87 (1896).
[3] Die Bewertung der Thomasmehl nach ihrem Gehalt am löslicher Phosphorsäure, Berlin, 1899.
[4] U. S. Dept. Agr. Bur. Chem. Bull. 152, 83 (1912).
[5] *This Journal*, 6, 254 (1923).
[6] *Ibid.*, 5, 97 (1921).
[7] *Ibid.*, 6, 259 (1923).
[8] *Ind. Eng. Chem. Anal. Ed.*, 3, 70 (1931).

and (3) the time of digestion. The change in solubility with the weight of the sample was shown to be of special significance in this determination. Thus the citrate-insoluble phosphoric acid in a number of ammoniated superphosphates having an ammonia content varying from 3.5 to 6.00 per cent was found to decrease on an average from 2.4 per cent to 1.1 per cent as the weight of the sample was decreased from 2 grams to 1 gram. Reducing the weight of the sample from 2 grams to 1 gram, or even to 0.5 gram, however, has no significant effect on the citrate-insoluble phosphoric acid in superphosphates that have not been ammoniated or otherwise reverted.

The average mixed fertilizer contains about 50 per cent of superphosphate, and the quantity of this material in a 2 gram sample of such a mixture will amount to only about 1 gram. When the phosphoric acid is supplied in the form of ammoniated superphosphate, analysis of a mixed fertilizer by the present official method will show a lower ratio of citrate-insoluble to total phosphoric acid than that found in the original ammoniated superphosphate, and the ratio will change in proportion as the ammoniated superphosphate in the mixture is changed. The method is therefore unsatisfactory when applied to this type of material, and numerous suggestions have been made for its improvement. The true availability of a material, however, can only be determined by vegetative tests, and such tests must necessarily precede the adoption of any proposed change in the laboratory method for measuring its availability. This paper gives a report on a collaborative study made with pot tests on the availability of the portion of the reverted phosphoric acid in ammoniated superphosphates that dissolves in ammonium citrate solution when the weight of the sample taken for analysis is reduced from 2 grams to 0.5 gram.

MATERIALS USED IN POT TESTS

The materials used in this work were a superphosphate manufactured and ammoniated by the Davison Chemical Company and a superphosphate manufactured by G. Ober & Sons Company by a special process. The latter, which is sold under the trade name of Oberphos, was ammoniated by the DuPont Ammonia Corporation. The composition of these two ammoniated products and the variation of their citrate-insoluble phosphoric acid with the weight of the sample taken for analysis are given in Table 1.

The citrate-insoluble phosphoric acid in the superphosphate from which the ammoniated superphosphate was prepared amounted to 0.25 per cent and that in the original Oberphos, to 1.1 per cent. These values deducted from the corresponding values in Table 1 show that about three-fourths of the reverted phosphoric acid in each of the ammoniated products goes into solution when the weight of the sample is reduced from 2 grams to 0.5 gram.

TABLE 1.

Variation in citrate-insoluble P_2O_5 in ammoniated phosphates with the weight of sample taken for analysis.

MATERIAL	AMMONIA (NH_3)	PHOSPHORIC ACID (P_2O_5)					
		WATER-INSOLUBLE	CITRATE-INSOLUBLE				TOTAL
			Wt. of sample taken for analysis				
			0.5 g.	1.0 g.	1.5 g.	2.0 g.	
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Ammoniated superphosphate	5.76	11.03	1.02	1.70	2.67	3.66	18.67
Ammoniated Oberphos	4.97	13.67	2.95	5.01	6.24	7.15	19.54

The procedure adopted for determining the true availability of this "soluble" portion of the reverted phosphoric acid in these ammoniated products consisted in making comparative vegetative tests on the two sets of citrate-insoluble residues prepared by washing each material with water and digesting one-half hour with ammonium citrate solution on the basis of (1) 2 grams per 100 cc. as directed in *Methods of Analysis, A. O. A. C.*, to give Residue A; and (2) one-fourth this quantity, or 0.5 gram per 100 cc. of solution, to give Residue B.

In preparing a sufficient quantity of these residues for use in the tests 500 gram portions of each material were washed by suction with 62.5 liters of cold water, and the washed residue was then transferred at once to the proper proportion of the citrate solution at 65°C. in a large enamel-lined, steam-jacketed kettle provided with a mechanical stirrer. The insoluble residue remaining at the end of the digestion was separated from the solution by means of 24 Pasteur-Chamberland filters, washed 7 or 8 times with

TABLE 2.

Phosphoric acid in citrate-insoluble residues from 1000 grams of original phosphate.

RESIDUE	WEIGHT	P_2O_5 CONTENT		P_2O_5 REVERTED	P_2O_5 ON BASIS OF TOTAL IN ORIGINAL MATERIAL
		TOTAL	REVERTED ON AMMONIATION		
	grams	*per cent*	*per cent*	*per cent*	*per cent*
Ammoniated superphosphate Residue A	129.20	19.41	17.48	90	13.43
Ammoniated superphosphate Residue B	89.29	12.93	10.12	78	6.18
Ammoniated Oberphos Residue A	238.30	27.04	22.43	83	32.98
Ammoniated Oberphos Residue B	149.26	21.08	13.71	65	16.10

water at 65°C. until the washings totaled 250 cc. per 2 grams of the original phosphate, and finally dried at 90°C.

The weights of the residues obtained per 1000 grams of material and their phosphoric acid (P_2O_5) content are given in Table 2.

The following phosphatic materials were also included with the samples that were submitted to each collaborator for use in pot tests.

MATERIAL	AVAILABLE P_2O_5	CITRATE-INSOLUBLE P_2O_5	TOTAL P_2O_5
	per cent	*per cent*	*per cent*
Monocalcium phosphate, $Ca(H_2PO_4)_2$	55.02		55.02
Dicalcium phosphate, $CaHPO_4$	48.43	2.01	50.44
Tricalcium phosphate, $Ca_3(PO_4)_2$	10.28	31.17	41.45
Tennessee brown rock phosphate	1.46	32.03	33.49
Ammoniated Oberphos	12.39	7.15	19.54

The ammoniated Oberphos and monocalcium phosphate were ground to pass a 20-mesh sieve, and the other materials to pass a 100-mesh sieve. The mechanical analysis of the Tennessee brown rock phosphate used in the test was as follows: coarser than 50 microns, 22.1 per cent; 50–5 microns, 60.1 per cent; finer than 5 microns, 17.8 per cent; finer than 2 microns, 13.4 per cent.

DIRECTIONS FOR POT TESTS

The choice of soil, crop and procedure for making the tests was left with each collaborator, but the following treatment was suggested for testing the availability of the so-called "soluble" portion of the P_2O_5 in Residue A.

Treatment No.	*Fertilizer Treatment*
1	N-K-No P
2	N-K-Residue B equivalent to Residue A in treatment No. 3.
3	N-K-Residue A containing x-gram of P_2O_5 that becomes soluble in the preparation of Residue B.
4	N-K-Residue B + x gram of P_2O_5 as monocalcium phosphate
5	N-K-Residue B + x gram of P_2O_5 as dicalcium phosphate

For example, if the phosphate applications are to be made at the rate of the equivalent of 500 pounds of 20 per cent superphosphate per acre and the pots have a capacity of 10 kg., then $x = 0.5$, and the quantities of each residue that should be taken in the proposed treatments should be as given in Tables 3 and 4.

If the capacity of the pots and the fertilizer applications differ from those cited, then corresponding changes should be made in the quantities of the phosphatic materials taken for the pot tests.

The following collaborators from the State agricultural experiment stations: S. D. Conner, Indiana; D. R. Hoagland, California; R. P. Bar-

tholomew, Arkansas; J. W. Tidmore, Alabama; and H. D. Haskins, Massachusetts; and F. R. Reid from the U. S. Department of Agriculture submitted reports on pot tests with the materials used in this investigation.

TABLE 3.

Treatment with ammoniated superphosphate residues.

TREATMENT NO.	PHOSPHATE MATERIAL REQUIRED					
	UNAVAILABLE			AVAILABLE		
	Material	Quantity	P_2O_5 in Material	Material	Quantity	P_2O_5 in Material
		gram	*gram*		*gram*	*gram*
1						
2	Residue B	3.302	0.427			
3	Residue A	4.775	0.427			0.50
4	Residue B	3.302	0.427	$Ca(H_2PO_4)_2$	0.9088	0.50
5	Residue B	3.302	0.427	$CaHPO_4$	1.0324	0.50

TABLE 4.

Treatment with ammoniated Oberphos residues.

TREATMENT NO.	PHOSPHATE MATERIAL REQUIRED					
	UNAVAILABLE			AVAILABLE		
	Material	Quantity	P_2O_5 in Material	Material	Quantity	P_2O_5 in Material
		gram	*gram*		*gram*	*gram*
1						
2	Residue B	2.264	0.477			
3	Residue A	3.613	0.477			0.50
4	Residue B	2.264	0 477	$Ca(H_2PO_4)_2$	0.9088	0.50
5	Residue B	2.264	0.477	$CaHPO_4$	1.0324	0.50

A tabulation of the results reported by four of the six collaborators is given in Table 5.

The results reported by Conner who applied each material separately in proportion to its total P_2O_5 content are given in Table 6. The plants were grown in 2 gallon pots of Bedford silt loam. All pots received 0.3 gram total P_2O_5 except No. 11, which received double this quantity. One gram of nitrogen and 1.5 gram of K_2O were also added to each pot in the form of ammonium nitrate and potassium chloride.

The tests made by Haskins included only a limited number of the samples. Each material was applied separately, as in Conner's experiments, in proportion to its P_2O_5 content. The plants were grown in pots containing 36 lbs. of air-dried soil that had received yearly applications of nitrogen, potash and lime but no phosphoric acid since 1890. The phosphatic materials used in the tests were applied at two rates of 1.143 grams and double this quantity of total P_2O_5 per pot. Each pot also received

TABLE 5.

Dry weight of crops in grams.

PHOSPHATE TREATMENT	AGRICULTURAL EXPERIMENT STATION OF									DEPARTMENT OF AGRICULTURE[4]		
	CALIFORNIA[1]			ARKANSAS[2]			ALABAMA[3]					
	Tomato plants	Increase over Check	Increase over Residue B	Sudan grass	Increase over Check	Increase over Residue B	Sorghum	Increase over Check	Increase over Residue B	Millet	Increase over Check	Increase over Residue B
Check	3 7			19 35			0 2			13 36		
Ammoniated superphosphate Residue B	11 8	8 1		26 33	6 98		56 8	56 6		13.98	0.62	
Ammoniated superphosphate Residue A	27 2	23 5	15 4	30 32	10 97	3 99	79 2	79 0	22.4	16.33	2.97	2.35
Ammoniated superphosphate Residue B +$Ca(H_2PO_4)_2$	34.7	31 0	22 9	41 23	21 88	14 90	108 1	107 9	51.3	17.13	3.77	3.15
Ammoniated superphosphate Residue B +$CaHPO_4$	19 0	15 3	7 2	39 51	20 16	13.18	69 6	69.4	12 8	17.71	4.35	3.73
Ammoniated superphosphate Residue B +$Ca_3(PO_4)_2$							66 4	66.2	9.6			
Ammoniated Oberphos Residue B	14 5	10.8		28.82	9 47		60 0	59.8		12 46	−0.90	
Ammoniated Oberphos Residue A	22 2	18.5	7 7	29 02	9.67	0 20	61.1	60 9	1.1	16 31	2.95	3.85
Ammoniated Oberphos Residue B +$Ca(H_2PO_4)_2$	30 5	26 8	16 0	38 83	19.48	10 01	75 8	75.6	15 8	16.93	3 57	4.47
Ammoniated Oberphos Residue B +$CaHPO_4$	22 2	18 5	7.7	37 82	18 47	9 00	77 9	77 7	17 9	17.06	3 70	4.60
Ammoniated Oberphos Residue B +$Ca_3(PO_4)_2$							67 6	67.4	7 6			
Monocalcium phosphate				34.83[5]	15 48		63 1[5]	62.9		15.56[6]	2 20	
Dicalcium phosphate							55.4[5]	55 2		14.28[6]	0 92	
Tricalcium phosphate							53 7[5]	53 5		13 41[6]	0 05	
Ammoniated Oberphos				33 83[5]	14 48					14 89[6]	1 53	

[1] Grown in triplicate in 8 kg of Aitkin clay soil
[2] Grown in triplicate in 7½ kg of Clarksville silt loam.
[3] Grown in duplicate in 7 kg of Cecil clay soil
[4] Grown in triplicate in 4½ kg of Lordstown silt loam, Steuben Co., N. Y
[5] P_2O_5 applied at rate of 0 5 gram per 10 kg of soil
[6] P_2O_5 applied at rate of 0 4 gram per 10 kg of soil.

16.33 grams of finely ground limestone, 35.76 grams of dried blood, 10.54 grams of nitrate of soda, 15 grams of sulfate of potash-magnesia, 8.2 grams of sulfate of potash and 16.33 grams of muriate of potash. The results are given in Table 7.

TABLE 6.

Dry weight of crop in grams.

(Indiana Agricultural Experiment Station)

TREATMENT NO.	PHOSPHATE TREATMENT	PHOSPHATE PER POT	BARLEY GRAIN AND STRAW	INCREASE OVER CHECK
		grams	*grams*	*grams*
1	None		7.0	
2	Ammoniated superphosphate Residue A	1.55	18.0	11.0
3	Ammoniated superphosphate Residue B	2.33	13.8	6.8
4	Ammoniated Oberphos Residue A	1.11	17.8	10.8
5	Ammoniated Oberphos Residue B	1.42	16.5	9.5
6	Monocalcium phosphate	0.55	21.0	14.0
7	Dicalcium phosphate	0.60	20.0	13.0
8	Tricalcium phosphate	0.72	18.5	11.5
9	Tennessee brown rock phosphate	0.90	12.5	5.5
10	Ammoniated Oberphos	1.53	18.5	11.5
11	Monocalcium phosphate	1.10	22.0	15.0

TABLE 7.

Dry weight of crop in grams.

(Massachusetts Agricultural Experiment Station)

TREATMENT NO.	PHOSPHATE TREATMENT	PHOSPHATE PER POT	DWARF ESSEX RAPE	INCREASE OVER CHECK
		grams	*grams*	*grams*
1	None		83.1	
2	Superphosphate (16 per cent)	7.14	119.6	36.5
3	Superphosphate (16 per cent)	14.28	153.8	70.7
4	Ammoniated Oberphos Residue A	4.23	116.5	33.4
4	Ammoniated Oberphos Residue A	8.46	159.5	76.4
6	Ammoniated Oberphos Residue B	5.42	98.4	15.3
7	Ammoniated Oberphos Residue B	10.84	114.7	31.6
8	Tennessee brown rock phosphate	3.26	77.5	−5.6
9	Tennessee brown rock phosphate	6.56	92.8	9.7

The results given in Tables 5, 6 and 7 are consistent throughout and show (1) that the B residues produced a marked increase in the growth of the crop over the check in most of the tests; (2) that the reverted portion of the phosphoric acid in the A residues is about 75 per cent as available, on an average, as that in monocalcium phosphate; (3) that dicalcium phosphate has about the same availability as monocalcium phosphate, giving

better results in some of the tests but not so good in others; and (4) that tricalcium phosphate was less available than the mono compound in all the tests in which it was used.

At the time the residues were prepared little was known regarding their composition or the reactions involved in the ammoniation of superphosphates. It was considered that the phosphoric acid in the B residues would have little or no availability, and it was to offset any specific effect that these residues might have on plants that the treatment outlined in Table 5 was suggested. The tests indicate that they had no special effect on plants apart from their P_2O_5 content, and as it is now known that they are more available than was at first anticipated, a better treatment to follow in the future is that in which all materials were applied separately in proportion to their total plant food content.

COMPOSITION OF CITRATE-INSOLUBLE RESIDUES

It has recently been shown by Jacob, Hill, Ross, and Rader[1] and by Keenen[2] that the reactions involved in the ammoniation of superphosphates vary with the quantity of ammonia added and the conditions under which the reaction takes place. The products of the reaction when ammonia is added up to about 2 per cent of the superphosphate, or to one mol equivalent of the monocalcium phosphate present, are mainly water- and citrate-soluble, as indicated in the following equation:

$$Ca(H_2PO_4)_2+NH_3=CaHPO_4+NH_4H_2PO_4$$

As more ammonia is added a greater or less proportion of the dicalcium phosphate is changed to tricalcium phosphate with formation of more ammonium phosphate. The latter reacts in turn in the presence of an excess of ammonia with the gypsum in the superphosphate to form ammonium sulfate and more dicalcium phosphate with continuous progression of the reactions as the absorption of ammonia is continued. The combined reactions may then be represented as follows:

$$Ca(H_2PO_4)_2+2CaSO_4+4NH_3=Ca_3(PO_4)_2+2(NH_4)_2SO_4$$

The reactions represented by this equation do not go to completion, however, under present operating conditions and a superphosphate having an ammonia content of 6 per cent or more will still contain water-soluble phosphoric acid. Other reactions varying with the temperature and moisture present will also occur. Thus a small portion of the tricalcium phosphate formed may undergo hydrolysis with formation of calcium hydroxyphosphate.

$$10\ Ca_3(PO_4)_2+6H_2O=3[3(Ca_3(PO_4)_2)\cdot Ca(OH)_2]+2H_3PO_4$$

[1] *Ind. Eng. Chem.*, 22, 1385 (1930).
[2] *Ibid.*, 22, 1378 (1930).

An ammoniated superphosphate may thus contain some ammonium phosphate, such iron and aluminum phosphates as may be present, and the following five calcium phosphates:

Monocalcium phosphate	$Ca(H_2PO_4)_2$
Dicalcium phosphate	$CaHPO_4$
Tricalcium phosphate	$Ca_3(PO_4)_2$
Calcium hydroxyphosphate	$3(Ca_3(PO_4)_2) \cdot Ca(OH)_2$
Undecomposed phosphate rock	$3(Ca_3(PO_4)_2) \cdot CaF_2$

The solubility of these compounds decreases in descending order.

An ammoniated superphosphate having 2 per cent of ammonia uniformly absorbed throughout the entire mass will contain mono- and dicalcium phosphates with little or no tricalcium phosphate; one having 4 per cent of ammonia will contain di- and tricalcium phosphates with little or no monocalcium phosphate, and one having the maximum of 6 per cent of ammonia will consist largely of tricalcium phosphate with possibly some calcium hydroxyphosphate but little or no mono- and dicalcium phosphates.

The residue obtained when an ammoniated superphosphate containing 4–6 per cent of ammonia is extracted with water and ammonium citrate solution on the basis of 2 grams per 100 cc. will, therefore, contain its phosphoric acid largely in the form of tricalcium phosphate together with smaller proportions of the hydroxyphosphate, iron and aluminum phosphates and undecomposed rock. If the extraction is made on the basis of 0.5 gram per 100 cc. of citrate solution, about three-fourths of the tricalcium phosphate in the original residue will go into solution, a portion will hydrolyze to the hydroxyphosphate, and the residue will then consist of the same compounds as before with a smaller proportion of the tricalcium phosphate and a larger proportion of the hydroxyphosphate.

The A residues from the ammoniated superphosphate and Oberphos used in this work contained 10 per cent and 17 per cent, respectively, of their total P_2O_5 in the form of iron and aluminum phosphates and undecomposed rock, while the same constituents supplied, respectively, 22 per cent and 35 per cent of the total P_2O_5 in the corresponding B residues. The reverted phosphoric acid in the A residues, consisting principally of tricalcium phosphate, amounted, therefore, to 90 and 83 per cent of the total, and that in the B residues, consisting of both tricalcium and calcium hydroxyphosphates, to 78 and 65 per cent, respectively.

It would, therefore, be expected (1) that the Tennessee brown rock phosphate consisting mainly of the least soluble of the calcium phosphates occurring in ammoniated superphosphates would be less available than either of the B residues; (2) that the B residues would be less available than the A residues; and (3) that the latter, containing a small portion of their phosphoric acid in a form less soluble than tricalcium phosphate

would have an availability slightly below that of pure tricalcium phosphate. Table 6 shows that the availability of the phosphoric acid in these materials found by pot tests is without exception in the order indicated by their composition.

The tests in which a direct comparison was made between mono- and tricalcium phosphate indicate that the latter is about 75 per cent as available as the former. This is in approximate agreement with data compiled by Parker[1] and by Richardson[2] on the availability of tricalcium phosphate and of materials of similar solubility, such as bone meal.

It would seem, therefore, that the simple procedure of reducing the weight of the sample taken for analysis in the ammonium citrate method from 2 grams to 0.5 gram would give a true measure of the availability of ammoniated superphosphates. It has also been found that a 75 per cent availability of these materials may be indicated by simply reducing the quantity of phosphate taken for analysis to 1 gram and extending the time of digestion from one-half to one hour. A 1 gram sample of ammoniated superphosphate is equivalent in P_2O_5 content to 2 grams of the average mixed fertilizer and the adoption of this procedure would call for a 2-gram sample as used at present in the analysis of all materials containing 10 per cent or less of total P_2O_5. A one-half hour digestion does not allow sufficient time for an ammoniated superphosphate to reach equilibrium with the ammonium citrate solution and slight differences in the time taken for digesting and filtering produce wide differences in the results. The values obtained when the digestion is extended to 1 hour are much more concordant, and this change in the official method with a reduction in the weight of the sample taken for analysis to 1 gram in the case of materials containing more than 10 per cent of total P_2O_5 thus seems to offer advantages over the procedure of digesting 0.5 gram for one-half hour.

These proposed changes in the official method offer the further advantage that they eliminate the necessity of a separate method for measuring the availability of precipitated phosphates. The accompanying paper by Jacob, Beeson, Rader, and Ross (p. 263) shows that the effects of the proposed changes are largely limited to superphosphates that have been treated with ammonia or otherwise reverted, and are of little significance when applied to phosphate rock and other constituents of mixed fertilizers such as iron and aluminum phosphates.

It is therefore recommended—[3]

(1) That the words, "Place 2 grams of the sample on a 9 cm. filter" in the official gravimetric method for the determination of water-soluble phosphoric acid (*Methods of Analysis, A. O. A. C.*, 1925, p. 4, sec. **11**, line 1) be changed to read—"Place 2 grams of the sample containing 10 per

[1] Private communication.
[2] Paper presented at the Cincinnati Meeting of the American Chemical Society, Sept 8–12, 1930
[3] For report of Subcommittee A and action of the association, see *This Journal*, **14**, 45 (1931).

cent or less of total phosphoric acid on a 9 cm. filter (if the sample contains more than 10 per cent of total phosphoric acid take 1 gram)."

(2) That the words, "treat 2 grams of the phosphatic material" in the official method for the determination of citrate-insoluble phosphoric acid in non-acidulated samples (*Methods of Analysis, A. O. A. C.*, 1925, p. 5, sec. **14 (b)**, line 2) be changed to read—"Treat 1 gram of the phosphatic material."

(3) That the words, "At the expiration of exactly 30 minutes" in the official method for the determination of citrate-insoluble phosphoric acid in acidulated samples (*Methods of Analysis, A. O. A. C.*, 1925, p. 5, sec. **14 (a)**, line 9) be changed to read—"At the expiration of 1 hour."

(4) That the collaborative study of the availability of superphosphates that have been treated with ammonia or other alkaline material be continued.

Note: The second, third, and fourth recommendations were adopted by the association unchanged. The wording of the first recommendation as adopted by the association is as follows:

1. That the words, "Place 2 grams of the sample on a 9 cm. filter" in the official gravimetric method for the determination of water-soluble phosphoric acid (*Methods of Analysis, A. O. A. C.*, p. 4, sec. **11**, line 1) be changed to read—"Place 1 gram of the sample on a 9 cm. filter."

SUPPLEMENTARY REPORT

The following results and comments submitted by E. Truog of the Wisconsin Agricultural Experiment Station were received too late to be included in this report as presented at the annual meeting of this association.

COMMENTS

The test was conducted in the greenhouse during the summer of 1930. Sudan grass was grown in two gallon glazed earthenware pots filled with pure quartz sand. A nutrient solution containing manganese and iodine and complete excepting for phosphorus was added to all the pots. The phosphates were added as directed. The Sudan grass grew normally and very well wherever sufficient available phosphate was present indicating favorable conditions for growth.

It has been known for a long time that ammonium salts increase the availability of tricalcium phosphate. This increased availability is probably due to at least two reasons. First, the nitrification of ammonium sulfate gives rise to two acids, nitric acid and sulfuric acid, which dissolve and make available the phosphate. Second, the efficiency of carbon dioxide as a solvent for tricalcium phosphate is increased by the presence of ammonium salts.

In order to take into consideration the influence of ammonium salts on the availability of tri-calcium phosphate, two nutrient solutions as indicated in the tabulated results were used, one free of ammonium salts and the other containing half of the nitrogen as ammonium sulfate. Unless this factor is considered in testing out Residues A and B, an incorrect conclusion might be drawn. In residues A and B, the ammonium salts naturally present in the original material have been removed and the availability of the phosphate in residues A and B is not what it is in the original material where ammonium salts are present.

TABLE 8.

Dry weight of crop in grams.

TREATMENT NO.	PHOSPHATE TREATMENT	FORM OF NITROGEN	SUDAN GRASS		
			Tops	Roots	Total
1	None	$KNO_3+Ca(NO_3)_2$	0.78	1.00	1.78
2	Ammoniated superphosphate Residue B	$KNO_3+Ca(NO_3)_2$	0.40	0.56	0.96
3	Ammoniated superphosphate Residue A	$KNO_3+Ca(NO_3)_2$	0.45	0.51	0.96
4	Ammoniated superphosphate Residue B+$Ca(H_2PO_4)_2$	$KNO_3+Ca(NO_3)_2$	25.35	11.45	36.80
5	Ammoniated superphosphate Residue B+$CaHPO_4$	$KNO_3+Ca(NO_3)_2$	27.85	11.47	39.32
6	Monocalcium phosphate, $Ca(H_2PO_4)_2$	$KNO_3+Ca(NO_3)_2$	26.55	11.45	38.00
7	Dicalcium phosphate, $CaHPO_4$	$KNO_3+Ca(NO_3)_2$	26.30	10.50	36.80
8	Tricalcium phosphate, $Ca_3(PO_4)_2$	$KNO_3+Ca(NO_3)_2$	2.13	2.21	4.34
9	Tricalcium phosphate, $Ca_3(PO_4)_2$	$(NH_4)_2SO_4+Ca(NO_3)_2$	34.65	12.87	47.52
10	Ammoniated Oberphos Residue B	$(NH_4)_2SO_4+Ca(NO_3)_2$	25.80	9.95	35.75
11	Ammoniated Oberphos Residue B	$KNO_3+Ca(NO_3)_2$	0.64	0.86	1.50
12	Ammoniated Oberphos Residue A	$KNO_3+Ca(NO_3)_2$	0.36	0.51	0.87
13	Ammoniated Oberphos Residue B+$Ca(H_2PO_4)_2$	$KNO_3+Ca(NO_3)_2$	26.58	11.27	37.85
14	Ammoniated Oberphos Residue B+$CaHPO_4$	$KNO_3+Ca(NO_3)_2$	27.30	11.40	38.70
15	Ammoniated Oberphos (untreated)	$KNO_3+Ca(NO_3)_2$	38.20	14.55	52.75
16	Tennessee brown rock phosphate	$KNO_3+Ca(NO_3)_2$	1.22	1.48	2.70
17	Tennessee brown rock phosphate	$(NH_4)_2SO_4+Ca(NO_3)_2$	16.55	8.02	24.57

This fact is brought out strikingly by the results given in the table. In other words, when superphosphate is ammoniated, we make the phosphate less available in one respect in that the phosphate is changed to a less soluble form, but we must not forget that we introduce something, namely, ammonium sulfate, which counteracts the reduction in solubility by producing an acid through nitrification which in the soil dissolves and changes the phosphate over to a soluble form. We must also remember that in the soil the conditions are different than in quartz cultures. Most soils have the capacity to take up bases, which in a sense has the same influence on the availability of the tricalcium phosphate as ammonium salts.

REPORT ON NITROGEN

By A. L. PRINCE (Agricultural Experiment Station,* New Brunswick, N. J.), *Associate Referee*

The Robertson method[1] for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide or urea was adopted as a tentative method last year. It was also recommended that further study be made on this method with a view to securing improvement. The work on the Robertson method for the past two years was carried out upon samples which contained no more than 200 pounds of nitrate per ton. The combined mineral and organic nitrogen was 6.35 per cent in one sample and 4.42 per cent in the other. In the analyses two grams of ferrous sulfate was used to combine with and drive off the nitrate nitrogen, which was subsequently determined by a method of difference.

Recently it was observed that when the combined nitrate and organic nitrogen was increased much beyond 5 or 6 per cent, the 2 grams of ferrous sulfate used in the analysis was insufficient. Theoretically, the quantity of nitrogen remaining after the digestion with ferrous sulfate should be equal to that originally present in the water-soluble form minus the nitrate nitrogen. Preliminary tests carried out by R. S. Gifford of the American Cyanamid Company and by the associate referee showed that when only 2 grams of ferrous sulfate was used, this amount of nitrogen was always less, and the loss increased as the amount of organic nitrogen and nitrates was increased in the mixtures. It was found, however, that this loss could be eliminated by increasing the amount of ferrous sulfate used. The problem that remained was to find out how much ferrous sulfate would be necessary to take care of mixtures high in nitrate and organic nitrogen as well as those low in these substances.

Hence, the object of this year's collaborative work was to study the use of various amounts of ferrous sulfate in connection with the Robertson method. This was attempted with the idea of improving upon the accuracy of the method, especially when large amounts of nitrate and organic nitrogen are present in the fertilizer mixtures.

* Journal Series paper of the New Jersey Agricultural Experiment Station, Department of Soil Chemistry and Bacteriology.

[1] *This Journal*, 13, 209 (1930).

The collaborative data upon last year's samples,[1] which contained 200 pounds of nitrate of soda per ton, indicated that 2 grams of ferrous sulfate was sufficient. This year two samples were prepared, each of which contained 500 pounds of nitrate of soda per ton. Sample No. 1 contained 250 pounds of urea per ton, while sample No. 2 contained 250 pounds of cyanamide per ton instead of the urea. The mixtures were prepared as follows:

	PARTS PER 100		NITROGEN BY ANALYSIS	NITROGEN IN SAMPLE—CALCULATED	
	No. 1	No. 2		No 1	No 2
			per cent	*per cent*	*per cent*
Urea	12 5		46.58	5.82	
Sodium nitrate	25.0	25.0	16.20	4.05	4.05
Calcium cyanamide		12.5	21.36		2.67
Potassium chloride	10.0	10.0			
Superphosphate	52.5	52.5			
	100.0	100.0		9.87	6.72

The samples were sent out to twelve chemists, and ten reports were received.

INSTRUCTIONS TO COLLABORATORS

EXPERIMENTS

Series I. Run each sample in duplicate by the Robertson method as described below.

Series II. Repeat part 4 of the Robertson method on each sample but use 5 grams of ferrous sulfate instead of 2 grams.

Series III. Repeat part 4 of the Robertson method on each sample but use 10 grams of ferrous sulfate instead of 2 grams.

Precaution: In Series II and III, where 5 and 10 grams of ferrous sulfate are used, severe bumping will occur during the evaporation of the water. The addition of 10 to 15 glass beads will help considerably to control bumping. After the water is evaporated, the bumping will diminish.

In the distillation process be sure to add a pinch of a mixture of zinc dust and granular zinc (20 mesh) in addition to the glass beads already present.

ROBERTSON METHOD

1. Determine the total nitrogen by the usual methods modified to include nitrates.

2. Weigh out 2 grams of the fertilizer mixture, wash to 200 cc., and determine the nitrogen in the residue by any of the modifications of the Kjeldahl methods. The difference between these two determinations gives the water-soluble nitrogen. (By weighing out duplicate 2.5 gram samples and washing to 250 cc. enough aliquots may be obtained to run the three series of experiments.)

3. Distil 50 cc. of the filtrate, equivalent to 0.5 gram, with magnesium oxide for ammoniacal nitrogen, as described in *Methods of Analysis, A.O.A.C.*, 1925, 11.

[1] *This Journal*, 13, 210 (1930).

4. Take another 50 cc. portion of the same solution, put into a 500 cc. Kjeldahl flask, together with 2 grams of ferrous sulfate and 20 cc. of 1.84 sp. gr. sulfuric acid. Digest over a hot flame. After the water is evaporated and white fumes appear, continue the digestion for at least 10 minutes. The nitrate nitrogen is thereby driven off. Add 0.65 gram of mercury and boil until all organic matter is oxidized. Cool, dilute, add potassium sulfide solution to precipitate the mercury, and distil with strong caustic soda in the usual way. A pinch of a mixture of zinc dust and granular zinc (20 mesh) should be added to each flask before distillation to prevent bumping.

1 (total) − 2 (water-insoluble) = water-soluble nitrogen.

The difference between the water-soluble nitrogen and the nitrogen obtained in 4 gives the nitrate nitrogen.

3 (ammonia nitrogen) + nitrate nitrogen gives total mineral nitrogen.

Total nitrogen less mineral nitrogen gives the organic nitrogen.

The collaborators were the following:

(1) R. S. Gifford, American Cyanamid Co., Linden, N. J.
(2) M. P. Etheredge, Mississippi State Chemical Laboratory, A. & M. College, Miss.
(3) A. H. Allen, Virginia-Carolina Chemical Corp., Richmond, Va.
(4) P. H. Emmett and R. Parrish, Bureau of Chemistry and Soils, Washington, D. C.
(5) H. D. Haskins and H. R. De Rose, Agricultural Experiment Station, Amherst, Mass.
(6) H. H. Hanson and R. E. Dickey, State Board of Agriculture, Dover, Del.
(7) A. C. Wark, Agricultural Experiment Station, New Brunswick, N. J.
(8) A. O. Olson, Dairy and Food Department, St. Paul, Minn.
(9) L. S. Walker and E. F. Boyce, Agricultural Experiment Station, Burlington, Vt.
(10) A. L. Prince.

The instructions to collaborators requested that three series of experiments be run on part 4 of the Robertson method. Each series varied in the amount of ferrous sulfate used, namely, 2, 5, and 10 grams.

The data from the collaborative study have been compiled in Tables 1, 2, 3, and 4; the figures are averages of duplicate or triplicate determinations. In the next to the last column is reported the deviation in nitrate nitrogen from the calculated value; the last column shows the deviation in organic nitrogen from the calculated value. At the bottom of each table are given the general averages of all the collaborators for each particular form of nitrogen determined, and the calculated values of the samples in question. In Table 4 is given a summary of the data showing the general average of all collaborative results.

DISCUSSION

The columns of figures in Table 1 should be compared with those corresponding columns in Tables 2 and 3. However, the results for the determinations of total, water-insoluble and water-soluble nitrogen and ammoniacal nitrogen are the same in each table, since there was no variation in the procedure and one set of determinations was sufficient.

TABLE 1.

Analysis of samples No. 1 and No. 2, 2 grams of $FeSO_4$ used.
(Results expressed as percentage of nitrogen.)

COLLABORATOR NO.	ROBERTSON METHOD 2 GRAMS $FeSO_4$ USED									
	Total nitrogen	Water-insoluble nitrogen	Water-soluble nitrogen	Nitrogen after $FeSO_4$ Treatment	Nitrate nitrogen	Ammoniacal nitrogen	Total mineral nitrogen	Organic nitrogen	Deviation in nitrate nitrogen from calculated value	Deviation in organic nitrogen from calculated value
					Sample 1					
1	9.95	0.01	9.94	5.32	4.62	0.30	4.92	5.03	+0.57	−0.79
2	9.81	0.02	9.79	5.63	4.16	0.45	4.61	5.20	+0.11	−0.62
3	9.80	0.01	9.79	5.64	4.15	0.22	4.37	5.43	+0.10	−0.39
4	9.86	0.03	9.83	5.18	4.66	0.41	5.07	4.79	+0.61	−1.03
5	9.90	0.05	9.85	5.40	4.45	0.41	4.86	5.04	+0.40	−0.78
6	10.01	0.04	9.97	5.66	4.31	0.40	4.71	5.30	+0.26	−0.52
7	9.83	0.01	9.82	5.58	4.24	0.34	4.58	5.25	+0.19	−0.57
8	9.78	0.07	9.71	5.44	4.27	0.22	4.49	5.29	+0.22	−0.53
9	10.06	0.03	10.03	5.50	4.53	0.38	4.91	5.15	+0.48	−0.67
10	9.88	0.00	9.88	5.16	4.72	0.27	5.09	4.79	+0.67	−1.03
Averages	9.89	0.03	9.86	5.45	4.41	0.34	4.76	5.13	+0.36	−0.69
Calculated values	9.87	0.00	9.87	5.82	4.05	0.00	4.05	5.82		
					Sample 2					
1	6.89	0.07	6.82	2.51	4.31	0.17	4.48	2.41	+0.43	−0.26
2	6.70	0.07	6.63	2.60	4.03	0.18	4.21	2.49	−0.02	−0.18
3	6.82	0.05	6.77	2.62	4.15	0.11	4.26	2.56	+0.10	−0.11
4	6.76	0.12	6.64	2.02	4.62	0.17	4.79	1.97	+0.57	−0.70
5	6.82	0.11	6.71	2.66	4.05	0.27	4.32	2.50	+0.00	−0.17
6	6.88	0.12	6.76	2.62	4.14	0.20	4.34	2.54	+0.08	−0.13
7	6.85	0.05	6.80	2.71	4.09	0.48	4.57	2.28	+0.04	−0.39
8	6.72	0.08	6.64	2.40	4.24	0.16	4.40	2.32	+0.19	−0.35
9	6.91	0.10	6.81	2.60	4.21	0.32	4.53	2.38	+0.16	−0.29
10	6.87	0.04	6.83	2.48	4.35	0.04	4.39	2.48	+0.30	−0.19
Averages	6.82	0.08	6.74	2.52	4.22	0.21	4.43	2.39	+0.19	−0.28
Calculated values	6.72	0.00	6.72	2.67	4.05	0.00	4.05	2.67		

TABLE 2.

Analysis of samples No. 1 and No. 2, 5 grams of $FeSO_4$ used.
(Results expressed as percentage of nitrogen.)

COLLABORATOR NO.	ROBERTSON METHOD 5 GRAMS $FeSO_4$ USED									
	Total nitrogen	Water-insoluble nitrogen	Water-soluble nitrogen	Nitrogen after $FeSO_4$ treatment	Nitrate nitrogen	Ammoniacal nitrogen	Total mineral nitrogen	Organic nitrogen	Deviation in nitrate nitrogen from calculated value	Deviation in organic nitrogen from calculated value
					Sample 1					
1	9.95	0.01	9.94	5.90	4.04	0.30	4.34	5.61	−0.01	−0.21
2	9.81	0.02	9.79	5.87	3.92	0.45	4.37	5.44	−0.13	−0.38
3	9.80	0.01	9.79	5.84	3.95	0.22	4.17	5.63	−0.10	−0.19
4	9.86	0.03	9.83	5.82	4.01	0.41	4.42	5.44	−0.04	−0.38
5	9.90	0.05	9.85	5.84	4.01	0.41	4.42	5.48	−0.04	−0.36
6	10.01	0.04	9.79	6.04	3.93	0.40	4.33	5.68	−0.12	−0.14
7	9.83	0.01	9.82	5.80	4.02	0.34	4.36	5.47	−0.03	−0.35
8	9.78	0.07	9.71	5.63	4.08	0.22	4.30	5.48	+0.03	−0.34
9	10.06	0.03	10.03	6.04	3.99	0.38	4.37	5.69	−0.06	−0.13
10	9.88	0.00	9.88	5.73	4.15	0.27	4.42	5.46	+0.10	−0.36
Averages	9.89	0.03	9.86	5.85	4.01	0.34	4.35	5.54	−0.04	−0.28
Calculated values	9.87	0.00	9.87	5.82	4.05	0.00	4.05	5.82		
					Sample 2					
1	6.89	0.07	6.82	2.73	4.09	0.17	4.26	2.63	+0.04	−0.04
2	6.70	0.07	6.63	2.66	3.97	0.18	4.15	2.55	−0.08	−0.12
3	6.82	0.05	6.77	2.74	4.03	0.11	4.14	2.68	−0.02	+0.01
4	6.76	0.12	6.64	2.60	4.04	0.17	4.21	2.55	−0.02	−0.12
5	6.82	0.11	6.71	2.78	3.93	0.27	4.20	2.62	−0.12	−0.05
6	6.88	0.12	6.76	2.92	3.84	0.20	4.04	2.84	−0.21	+0.17
7	6.85	0.05	6.80	2.83	3.97	0.48	4.45	2.40	−0.08	−0.27
8	6.72	0.08	6.64	2.62	4.02	0.16	4.18	2.54	−0.03	−0.13
9	6.91	0.10	6.81	2.86	3.95	0.32	4.27	2.64	−0.10	−0.03
10	6.87	0.04	6.83	2.67	4.16	0.04	4.20	2.67	+0.11	−0.00
Averages	6.82	0.08	6.74	2.74	4.00	0.21	4.21	2.61	−0.05	−0.06
Calculated values	6.72	0.00	6.72	2.67	4.05	0.00	4.05	2.67		

TABLE 3.

Analysis of samples No. 1 and No. 2, 10 grams of $FeSO_4$ used.
(Results expressed as percentage of nitrogen.)

COLLABORATOR	ROBERTSON METHOD 10 GRAMS $FeSO_4$ USED									
	Total nitrogen	Water-insoluble nitrogen	Water-soluble nitrogen	Nitrogen after $FeSO_4$ treatment	Nitrate nitrogen	Ammoniacal nitrogen	Total mineral nitrogen	Organic nitrogen	Deviation in nitrate nitrogen from calculated value	Deviation in organic nitrogen from calculated value
					Sample 1					
1	9.95	0.01	9.94	5 96	3.98	0.30	4.28	5.67	−0.07	−0.15
2	9.81	0 02	9.79	5.93	3.86	0.45	4.31	5.50	−0.19	−0.32
3	9.80	0.01	9 79	5.84	3.95	0.22	4.17	5.63	−0.10	−0.19
4	9.86	0.03	9 83	5.92	3.91	0.41	4.32	5.54	−0.14	−0.28
5	9.90	0.05	9.85	5.92	3 93	0.41	4 34	5.56	−0.12	−0.26
6	10 01	0 04	9.97	6.13	3.84	0.40	4 24	5.77	−0.21	−0.05
7	9.83	0.01	9.82	5 87	3.95	0.34	4.29	5.54	−0.10	−0.28
8	9.78	0.07	9.71	5 69	4.02	0.22	4.24	5 54	−0 03	−0.28
9	10 06	0.03	10 03	5.90	4.13	0 38	4.51	5.55	+0.08	−0.27
10	9.88	0.00	9.88	5 82	4.06	0.27	4 33	5.55	+0.01	−0.27
Averages	9.89	0.03	9.86	5.90	3 96	0 34	4.30	5.59	−0.09	−0.23
Calculated values	9.87	0.00	9.87	5.82	4.05	0 00	4.05	5.82		
					Sample 2					
1	6.89	0.07	6.82	2.75	4 07	0.17	4.24	2.65	+0.02	−0.02
2	6.70	0.07	6.63	2 71	3.92	0.18	4.10	2.60	−0.13	−0.07
3	6.82	0.05	6.77	2.74	4.03	0.11	4.14	2.68	−0.02	+0.01
4	6.76	0.12	6 64	2 41	4.23	0 17	4 40	2.36	+0 18	−0.31
5	6.82	0.11	6 71	2 86	3.85	0.27	4 12	2.70	−0 20	+0.03
6	6.88	0.12	6.76	2 93	3.83	0 20	4.03	2 85	−0.22	+0.18
7	6 85	0.05	6.80	2.85	3.95	0.48	4.43	2 42	−0.10	−0.25
8	6.72	0 08	6.64	2.71	3.93	0.16	4.09	2.63	−0.12	−0.04
9	6.91	0.10	6 81	2 76	4 05	0 32	4 37	2 54	0.00	−0.13
10	6 87	0 04	6 83	2 65	4.18	0.04	4 22	2 65	+0 13	−0.02
Averages	6.82	0.08	6.74	2 74	4 00	0 21	4.21	2.61	−0.05	−0 06
Calculated values	6.72	0 00	6.72	2.67	4.05	0.00	4.05	2.67		

TABLE 4.

Summary of data showing average of all collaborative results.

AMOUNT $FeSO_4$ USED	TOTAL NITROGEN	WATER-INSOLUBLE NITROGEN	WATER-SOLUBLE NITROGEN	NITROGEN AFTER $FeSO_4$ TREATMENT	NITRATE NITROGEN	AMMONIACAL NITROGEN	TOTAL MINERAL NITROGEN	ORGANIC NITROGEN	DEVIATION IN NITRATE NITROGEN FROM CALCULATED VALUE	DEVIATION IN ORGANIC NITROGEN FROM CALCULATED VALUE
					Sample 1					
2 grams	9.89	0.03	9.86	5.45	4.41	0.34	4.76	5.13	+0.36	−0.69
5 grams	9.89	0.03	9.86	5.85	4.01	0.34	4.35	5.54	−0.04	−0.28
10 grams	9.89	0.03	9.86	5.90	3.96	0.34	4.30	5.59	−0.09	−0.23
Calculated values	9.87	0.00	9.87	5.82	4.05	0.00	4.05	5.82		
					Sample 2					
2 grams	6.82	0.08	6.74	2.52	4.22	0.21	4.43	2.39	+0.19	−0.28
5 grams	6.82	0.08	6.74	2.74	4.00	0.21	4.21	2.61	−0.05	−0.06
10 grams	6.82	0.08	6.74	2.74	4.00	0.21	4.21	2.61	−0.05	−0.06
Calculated values	6.72	0.00	6.72	2.67	4.05	0.00	4.05	2.67		

The total nitrogen given in column 1 was run by the regular Kjeldahl method to include nitrates. The collaborative results for the total nitrogen content on both samples are in quite close agreement, the general average being 9.89 per cent as against 9.87 per cent for the calculated value. In columns 2 and 3 are reported the water-insoluble and water-soluble nitrogen. Theoretically, there should be no water-insoluble nitrogen in these two samples, but the actual analysis showed a small amount, averaging only 0.03 per cent in sample 1 and 0.08 per cent in sample 2. The determination of the water-soluble nitrogen, which is a necessary part in the Robertson scheme for the separation of nitrate nitrogen, showed good collaborative results.

In column 4 is recorded the nitrogen after the ferrous sulfate treatment. This determination is made on a portion of the water-soluble extract, and by treatment with ferrous sulfate the nitrate nitrogen is eliminated, but the rest of the water-soluble nitrogen remains. It is this portion of the water-soluble nitrogen that is reported in column 4. Theoretically, the value for this determination should be the same as the calculated value for the organic nitrogen on these particular samples, as they contained theoretically no water-insoluble nitrogen or ammonia nitrogen. The average percentage of nitrogen for column 4, sample 1, was 5.45, while the calculated value for the organic nitrogen was 5.82. With sample 2, the corresponding figures were 2.52 and 2.67 per cent. Thus there was an apparent loss of nitrogen after the ferrous sulfate treatment when only 2 grams of the latter was used. The loss appeared to be much greater in sample 1, which contained urea. In column 4, table 2, where 5 grams of ferrous sulfate was used, the average collaborative result came much closer to the calculated value on sample 1. On sample 2, the average result showed a slight gain over the calculated value, but the deviation was not excessive. The corresponding figures in table 3, where 10 grams were used, showed a slight gain in the nitrogen over the calculated values.

The nitrate nitrogen results are reported in column 5. With 2 grams of ferrous sulfate, the nitrate nitrogen figures were decidedly higher than the calculated value. The general average was 4.41 per cent as against the calculated value of 4.05 per cent. On sample 2 the deviation was in the same direction but of not such magnitude. With 5 grams of ferrous sulfate, the nitrate nitrogen figures were much closer to the calculated value, the average deviation on sample 1 being 0.04 per cent and 0.05 per cent on sample 2. When 10 grams of ferrous sulfate was used there appeared to be a slight loss of nitrate nitrogen although the average deviation from the calculated value was only 0.09 per cent on sample 1 and 0.05 per cent on sample 2. It is very interesting to note that with sample 2 the general average of all the collaborative results for the 5 and 10 gram portions of ferrous sulfate was exactly the same on all determinations. This may be clearly seen in Table 4.

Apparently there is no advantage gained by using 10 grams of the ferrous sulfate. It should be pointed out that for manipulative reasons the minimum amount of ferrous sulfate used should be consistent with obtaining accurate results. Large amounts of ferrous sulfate cause very severe bumping during the digestion, which of course may be overcome to a considerable degree by the use of glass beads. The 5 gram portion of ferrous sulfate appears to be sufficient to cover all cases and to secure accurate results for nitrate nitrogen. From a study of last year's collaborative data, it is believed that 2 grams of ferrous sulfate is sufficient on all samples that do not contain over 5 per cent total nitrogen. Therefore, the associate referee suggests that the procedure of the Robertson method be modified to include the use of 5 grams of ferrous sulfate in all cases where the total nitrogen content has been found to be over 5 per cent.

One of the weaknesses of the Robertson method, which is also true of the older methods, appears in the determination of the ammoniacal form of nitrogen, which is reported in column 6. Theoretically there was no ammoniacal nitrogen in either sample, but the average found was 0.34 per cent for sample 1 and 0.21 per cent for sample 2. This error is no doubt due to the action of magnesium oxide, which tends to break down the urea and cyanamide, in the distillation of the ammonia form of nitrogen. The urea is decomposed to a greater extent than the cyanamide by this procedure. The total mineral nitrogen consists of the nitrate and ammoniacal forms of nitrogen and the collaborative data for this combination is reported in column 7. These figures, of course, are much higher than the calculated values owing to the combined errors in the determination of the ammonia and nitrate forms of nitrogen. The organic form of nitrogen, which is obtained by subtracting the mineral nitrogen from the total nitrogen, is necessarily low, as may be seen in column 8. With sample 1, using 2 grams of ferrous sulfate, the average deviation from the calculated value for organic nitrogen was 0.69 per cent and for sample 2, 0.28 per cent. When 5 grams of ferrous sulfate was used to improve the nitrate nitrogen determination, the organic nitrogen results were somewhat nearer to the calculated value, but they were still low due to the error incurred in the determination of the ammonia form of nitrogen. Thus, by obtaining correct nitrate nitrogen values in the presence of urea and cyanamide, the Robertson method has also diminished the error ordinarily thrown onto the organic form of nitrogen.

However, with the continued use of urea in mixed fertilizers, it is the opinion of the associate referee that improvement in the differentiation between mineral and organic nitrogen should be sought in a better method for ammoniacal nitrogen determinations. An aspiration method, using alkali at low temperature, might be a means of obtaining more normal ammoniacal nitrogen results in the presence of urea, but such a method

has the disadvantage of consuming much time, and therefore it is undesirable for control work.

There is another matter that should be brought to the attention of the association at this time. In 1924 the Devarda alloy method for the determination of nitrate nitrogen in nitrate salts[1] was adopted tentatively, after two or three years of extensive collaborative study. Owing to an oversight or to the pressure of other work, this method was not presented for final action. Since that time the method has continued to meet with favor and has proved satisfactory for the purpose for which it was devised. In the light of these facts, the associate referee suggests that the Devarda method for the determination of nitrate nitrogen in nitrate salts be made official.

RECOMMENDATIONS[2]

It is recommended—

(1) That the Robertson method for the determination of nitrate nitrogen in mixed fertilizers containing cyanamide or urea be adopted as official, with the following addition to be incorporated in the proper place in the procedure: "Use 5 grams of ferrous sulfate instead of 2 grams if the total nitrogen is found to be over 5 per cent" (final action).

(2) That the Devarda method for the determination of nitrates in nitrate salts be made official (first action).

(3) That an attempt be made to devise a method practical for control work, which will determine accurately the ammoniacal nitrogen in the presence of urea and cyanamide.

REPORT ON NITROGEN ACTIVITY METHODS IN FERTILIZERS

By JOHN B. SMITH (Agricultural Experiment Station, Kingston, R. I.), *Associate Referee*

No work has been done on these methods during the past year. Attention is directed to changes in the alkaline permanganate method[3] recommended last year[4] prescribing the procedure for controlling the concentration of potassium permanganate in the digestion solution, and allowing the washed residue containing the water-insoluble nitrogen to be transferred while wet to the digestion flask.

Through an error in the original report the directions for the preparation of the stock solution of potassium permanganate as published in the report of Subcommittee A[5] require the solution to be adjusted to contain

[1] *This Journal*, 8, 263 (1925).
[2] For report of Subcommittee A and action of the association, see *This Journal*, 14, 46 (1931)
[3] *Methods of Analysis, A.O.A.C.*, 1925, 12.
[4] *This Journal*, 13, 215 (1930).
[5] *Ibid.*, 61.

2.5 grams per liter in place of the proper quantity, 50 grams. All other published writings of the report have been corrected.

It is recommended[1] that the editorial error noted above be corrected and that the changes approved last year (first reading) be adopted as final.

REPORT ON HIGH ANALYSIS FERTILIZERS[2]

By JOHN B. SMITH (Agricultural Experiment Station, Kingston, R. I.), *Associate Referee*

As a part of a study of the adaptation of the official methods for the analysis of the new fertilizer materials, usually more concentrated in plant food than the more familiar products, it is obviously important to know what methods have proved unsatisfactory to those interested. The Fertilizer Section of the American Chemical Society has voiced the general sentiment in a request for study of sampling, preparation of sample, and the determination of ingredients, especially nitrogen. Correspondence with manufacturers, distributors, and State officials elicited the following list of topics, both general and specific:

From manufacturers—

Loss of weight at 100°C. does not measure moisture in materials that decompose below that temperature. Vacuum drying at lower temperatures is required.

The official method for moisture does not give uniform results for calcium nitrate since of 100 reports of analysis only 6 fall below a guaranty of 15 per cent nitrogen, but the moisture results vary from 1.11 to 12.77 per cent. Actual dilution to this extent must have affected the percentage of nitrogen.

Distillation of technical ammonium phosphate with magnesium oxide does not recover all the ammonia. Distillation with an excess of caustic soda is required.

The Devarda method is superior to other methods for determining nitrogen in nitrates and should be made official.

The methods used commonly in America give lower results for nitrogen in Calurea, a mixture of urea, calcium nitrate, and ammonium nitrate, than does a modification used in Germany, but American analysis of mixtures containing Calurea give full credit to quantities of that ingredient mixed on the basis of German results.

The official method does not remove all the water-soluble phosphate from 2 gram samples of materials high in phosphate, (Ammophos, etc.).

From State Officials—

Moisture changes in hygroscopic materials cause variations in analysis, both before sampling and during preparation and weighing.

Factory mixing is not sufficiently uniform to allow accurate representation by small samples. This applies both to the sample of the shipment and to the portions weighed for analysis. Aliquots from solutions of large samples are more representative than small portions weighed separately if the materials are soluble. It has been noted particularly that many mixtures contain large crystals of muriate of potash

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 46 (1931).
[2] Contribution No. 402 of the Rhode Island Agricultural Experiment Station.

that have escaped grinding at the factory. Grinding the analytical sample very fine allows better representation by small weighed portions.

When bases forming insoluble phosphates are not present in sufficient quantities to precipitate the phosphates in water solutions of potash, more of such bases should be added before proceeding with the Lindo-Gladding technic.

Water held by hygroscopic salts may interfere with the efficiency of salicylic acid in holding nitrates for the determination of nitrogen.

All the suggestions listed seem pertinent. Certain of the topics are already under investigation by the associate referees who are studying methods for the individual elements, and others come quite definitely within those fields. Therefore it seemed best to confine the work this year to an investigation of certain physical characteristics that are intimately related to chemical analysis.

Hygroscopicity undoubtedly causes fluctuation in the moisture present in a number of the salts and mixtures and the changes affect sampling, preparation and manipulation of sample, and the percentages of the different elements found, as it is not the custom to correct results of fertilizer analyses for moisture changes.

Ross and his associates of the Division of Fertilizer and Fixed Nitrogen Investigations, Bureau of Chemistry and Soils, have published the results of a number of fundamental researches with regard to the relative hygroscopicity of various pure salts of a nature similar to those used in fertilizer mixtures.[1] They point out that there is a critical aqueous vapor pressure for each soluble salt, measured for any definite temperature by the aqueous vapor pressure of a saturated solution of the salt in a closed chamber. In humidities above this critical point the salt absorbs moisture, while in those below moisture is given up. Of the materials listed, calcium nitrate, ammonium nitrate, sodium nitrate, and urea are the most hygroscopic, while monoammonium phosphate and potash salts are the least. They have further shown that for a considerable number of materials a definite constant water content can be established for each individual salt if it is allowed to reach equilibrium with the moisture in an atmosphere of definite relative humidity and temperature. Increasing either the temperature or humidity increased the absorption of moisture.[2] Quinones[3] concluded that with the high temperature and humidity that prevail in Porto Rico, many fertilizer mixtures absorb sufficient moisture to cause analyses to fall below guaranties, and that excessive absorption near the surface of the sacks is a disturbing factor in sampling.

MOISTURE VARIATION IN SEVERAL HYGROSCOPIC MATERIALS

To see how great is the variation among different shipments of the same product, moisture was determined in as many samples of the more hygro-

[1] *Ind. Eng. Chem.*, 19, 211 (1927); 21, 305 (1929).
[2] *Ibid.*, 21, 1219 (1929).
[3] *Am. Fertilizer*, 72, No. 10, 19 (1930).

scopic fertilizer materials as could be secured by the cooperation of a number of the members of the association. Most of the materials came from official State samples, but when these proved too few, the collection was increased through the courtesy of officials of the American Synthetic Nitrogen Products Corporation, the samples being taken from different shipments in widely separated parts of the country.

The method used in the work as a standard is that of drying to constant weight at 30°C. in an evacuated desiccator containing barium perchlorate. This method was suggested tentatively by Albert R. Merz. The desiccant is claimed by the manufacturers to reduce the partial pressure of water vapor to 0.001 mm. of mercury, a desiccation comparable to that secured with phosphorous pentoxide and which is superior to sulfuric acid. Time did not allow for thorough tests of the method, but quantities of added water varying from 1 to 4 per cent were recovered from urea almost exactly, although nearly a week was required for recovery of the largest quantity. This method of desiccation should be more rapid and thorough than drying over sulfuric acid at 1 atmosphere pressure, as recommended by German workers for the products studied. All samples of each material were dried in the same desiccator and at the same time, so that results might be strictly comparable. For comparison, loss of weight upon drying at 100°C, 1 atmosphere pressure, was determined, but it is recognized that such losses are not all moisture. Of the commercial products studied, Calurea, urea, and Nitrophoska decompose below that temperature, while calcium nitrate loses a considerable portion of its water of crystallization, but probably not all.

In all cases approximately 5 gram samples were weighed rapidly into accurately tared weighing bottles 50 mm. in diameter. To avoid moisture changes in manipulation, the bottles were then closed and weighed accurately.The samples were unground for the most part, but a few had apparently been ground for analysis before reaching this laboratory.

Moistures found in 10 samples each of Nitrophoska No. 1, urea, and Calurea are reported in Table 1. The Nitrophoska reached equilibrium with the drying conditions—reduced pressure at 30°C. in the presence of barium perchlorate—after 96 hours; urea and Calurea, after 24 hours. The average moisture in 10 samples of urea was 0.09 per cent, and the greatest variation from the average was 0.20 per cent. This variation would cause a change of less than 0.1 per cent in the actual percentage of nitrogen in urea and is insignificant.

Nitrophoska contained an average of 1.66 per cent of moisture, and the maximum variation from the average was +1.31 per cent. This change is sufficient to reduce the percentage of P_2O_5 from 30 to 29.60. The average moisture for 11 samples of Calurea was 1.04 per cent, and the variation was much greater than for that of the products mentioned above. The

greatest deviation from the mean was +2.25 per cent, a sufficient dilution to reduce the percentage of nitrogen from 34 to 33.23.

The loss on drying at 100°C. in air was much greater for all the materials than that by the reduced pressure with low temperature, but it cannot be regarded as a measurement of moisture. A strong odor of ammonia was noted from Nitrophoska, and portions of both Nitrophoska and urea sublime when heated to 100°C. in closed dishes. Calurea is a mixture of urea, calcium nitrate, and a small proportion of ammonium nitrate. No sublimation was noted for this material, but the urea is probably decomposed in much the same manner as when heated alone.

TABLE 1.

Moisture in different shipments, determined by (A) loss under reduced pressure at 30°C. and in the presence of barium perchlorate, (B) drying at 100° C. in air.

(Results are expressed in percentage)

NITROPHOSKA NO 1		UREA		CALUREA		CALCIUM NITRATE	
A	B	A	B	A	B	A*	B
1.69	8.25	0.06	1.54	0.74	3.69	3.74	19.40
1 90	8.01	0.04	2.34	0.87	5.44	7 84	15 93
1.25	7.46	0.04	0.60	0.93	2.86	16 80	26 82
1.42	7.64	0.13	1.30	0 60	--	3.56	15.87
0.92	7.77	0.17	2 35	0.96	2.48	4 77	14.35
2.07	7.78	0 29	3 34	3 12	3.69	8 55	20 14
2 97	8.45	Trace	1.29	0.05	0.22	7.44	17 62
1.66	7.79	Trace	1 05	3.29	4 14	7 03	16.90
1.30	7.43	0.10	1.00	0.61	2.05	4 97	21 50
1.41	7.94	0.09	0.84	0.08	0.79		
				0.20	0.76		
Average		Average		Average			
1.66		0.09		1.04			

* Had not reached equilibrium

The results for calcium nitrate also appear in Table 1. From information furnished by representatives of the Synthetic Nitrogen Products Corporation, two types of calcium nitrate are manufactured in Europe. The Norwegian product is the trihydrate containing 26 per cent of water, and the German calcium nitrate is a mixture of dihydrate containing 18 per cent of water and the anhydrous salt. The mixture contains about12 per cent of water of crystallization. In addition, 4 per cent of ammonium nitrate is present. This presents the problem of separation of the free water from the water of crystallization, or the accurate determination of the total water without decomposition of the ammonium nitrate. Thus far no solution has been found for the problem. Drying at 100°C. gave very high results and may have caused decomposition, but it probably did not remove the water completely. Drying in vacuum at 40°C. or at 35°C. did

not produce equilibrium within a reasonable time. Drying in an evacuated desiccator at 35°C. for 144 hours gave results approaching equilibrium. The losses during the final 24 hour period averaged 0.18 per cent, and apparently equilibrium would soon have been reached had time allowed continuation of the work. The great discrepancy between these results and those from drying at 100°C. is disconcerting and leads to the inference that some definite degree of hydration exists when the material is in equilibrium with the drying conditions. Furthermore the degree of dryness at the outside of the crystals appeared quite different from that at the center.

The granules of calcium nitrate disintegrate in toluene but do not appear to dissolve. Possibly distillation from that reagent and measurement of the water in the distillate might prove an accurate method. Despite the inaccuracy of the determinations there is no doubt that a considerable variation in moisture exists among the different samples of calcium nitrate. One sample received carefully sealed from a State official was obviously wet and lost more than twice the moisture during desiccation at low temperature than was the average for the remainder of the samples.

In so far as the few samples studied represent conditions, the variations in moisture affect the analyses of Nitrophoska, Calurea, and calcium nitrate significantly.

UNIFORM MOISTURE CONDITIONS IN CALCIUM NITRATE

It was noted previously that at definite relative humidity and temperature, soluble salts gain or lose moisture according as the moisture present is below or above that which they possess when in equilibrium with the stated conditions. Thus, different samples of the same product should reach uniformity if subjected to the same atmospheric conditions for a sufficient time. Mehring[1] showed this to be true for calcium nitrate and other fertilizer salts if the materials are first ground to an equal degree of fineness. This fact has an application to the problem, because if it is easier to produce uniformity by this means than to determine moisture for the purpose of correcting results, the same object would be served.

In order to demonstrate the possibility, the 9 samples of commercial calcium nitrate were placed in an atmosphere with a relative humidity of 23 per cent, created by placing a solution saturated with both calcium nitrate and ammonium nitrate[2] in the bottom of a desiccator and holding this at a temperature of 30°C. in a constant temperature closet. Unfortunately the precaution of uniform grinding was not observed.

Despite differences in fineness the demonstration was relatively successful, as is shown by the data in Table 2. Sample 3, which was decidedly wet when received, lost 5.21 per cent of moisture; all other samples gained because the relative humidity was above that with which the materials were

[1] *Ind. Eng. Chem.*, 21, 1219 (1929).
[2] *Ibid.*, 305.

previously in equilibrium; a period of 14 days was required, however, before all the samples had ceased to gain in weight. Possibly the time might have been reduced by grinding and daily stirring. The samples were then dried at 30°C. under reduced pressure in the presence of barium perchlorate, as already noted, approximate equilibrium being reached after six days. The losses varied from 11.57 per cent to 13.86 per cent, with 7 of the 9 samples grouped between 12.92 per cent and 13.86 per cent. These results may be compared with losses varying from 3.74 per cent to 16.80 per cent for the same samples in the original state.

TABLE 2.

Creation of uniformity in the moisture content of different samples of calcium nitrate.

SAMPLE	GAIN OR LOSS IN WEIGHT AFTER 14 DAYS AT 30°C., 23 PER CENT RELATIVE HUMIDITY	MOISTURE AFTER* TREATMENT	MOISTURE* ORIGINAL SAMPLE
	per cent	*per cent*	*per cent*
1	+9.87	13.61	3.74
2	+5.08	12.92	7.84
3	−5.21	11.58	16.80
4	+9.49	13.05	3.56
5	+9.08	13.85	4.77
6	+5.31	13.86	8.55
7	+6 13	13.58	7.44
8	+2.61	11.57	7.03
9	+8.58	13.55	4.97

* Loss at 30°C. under reduced pressure in the presence of barium perchlorate.

This procedure presents distinct possibilities if the time required can be shortened. Selection of a lower relative humidity would correct the disadvantage of increasing the moisture above that normally present.

MOISTURE CHANGES DURING WEIGHING

To study moisture changes during weighing and the general manipulation requiring exposure to the air, samples weighing approximately 2 grams were poured in low conical heaps between tared watch-glasses. While thus protected from the air, the watch-glasses and samples were weighed accurately. A sample was then exposed in an open balance by removing the upper glass and placing it under the lower one. Weighings were made rapidly at definite time intervals. Weather conditions to give a high rate of absorption were selected; on the days chosen the relative humidity was between 85 and 90 per cent, as measured by the usual wet and dry bulb temperature method, and the air temperature was between 75° and 85°F. The results are reported in Table 3.

Of the materials studied calcium nitrate proved by far the most hygroscopic, and grinding increased the rate of absorption. Urea, Calurea, Cal-

nitro, synthetic nitrate of soda, and Nitrophoska No. 1, all absorbed appreciable quantities of water in a half-hour period. Ammophos B, Ammophosko No. 1, and No. 2 did not gain in weight.

The error is measured exactly by the percentage of gain or loss of water, but it appears large or small in accordance with the percentage of the element estimated. An increase of 2 per cent in the weight of calcium nitrate decreases the percentage of nitrogen from 15 to 14.70, while an increase of only 0.5 per cent for urea decreases the percentage of nitrogen from 46 to 45.77, nearly an equal amount.

TABLE 3.

Moisture absorption by a 2 gram sample exposed to air at a relative humidity of approximately 90 per cent and a temperature of approximately 80°F.

(Results are expressed in percentage.)

MATERIAL	MOISTURE ABSORBED						
	1 min.	5 min.	10 min.	15 min.	20 min.	25 min.	30 min.
Calcium nitrate*	0.14	0.43	0.79	1.07	1.39	1.66	1.94
Calcium nitrate†	0.10	0.43	0.81	1.16	1.52	1.86	2.22
Urea*	0.05	0.15	0.27	0.33	0.40	0.48	0.51
Calurea*	0.04	0.14	0.22	0.29	0.32	0.36	0.42
Calnitro*	—	0.06	0.12	0.16	0.19	0.21	0.27
Synthetic Nitrate of Soda*	—	0.06	0.08	0.10	0.14	0.15	0.16
Nitrophoska No.1†	—	0.06	0.08	0.10	0.10	0.12	0.13
Ammophosko No. 1†	—	0	0	0	0	0	0
Ammophosko No. 2†	—	0	0	0	0	0	0
Ammophos B†	—	0	0	0	0	0	0

* Unground.
† Ground.

In general, the absorption under approximately maximum conditions is not sufficient to cause serious error during the first 5 minutes, ample time for making a weighing, but the half hour often required for grinding would allow material dilution of calcium nitrate, urea, Calurea, and possibly of other products. Ammophos, and Ammophosko No. 1 and 2, take up water slowly, if at all, and apparently may be ground and weighed in air without error.

CONCLUSIONS

Moisture variations in the more hygroscopic fertilizer salts are sufficient to cause significant changes in the percentages of plant food elements during shipment, storage, and the customary procedure of analysis.

These changes may be corrected by calculation of the results of analyses to a uniform moisture basis, or, possibly, by subjection of samples to definite relative humidity and temperature for sufficient time to create uniformity.

The official method for the determination of moisture is not applicable to materials that decompose below the temperature of 100°C. Drying to constant weight at 30°C. in an evacuated desiccator containing an efficient desiccant seems a satisfactory method, but its adaptation to all materials has not been sufficiently established.

The rate of water absorption by the materials studied does not cause significant errors during the short time required for weighing samples for analysis, but exposure of the more hygroscopic products to a humid atmosphere for the length of time required for grinding causes material dilution.

RECOMMENDATIONS[1]

It is recommended that the subject of moisture variation be given further study, with a view to correction of analyses for such variation, and that a method for the determination of moisture be devised that is applicable to materials that decompose below a temperature of 100°C. It is further recommended that sampling methods, preparation of sample, and methods for securing a proper portion of the sample for analysis be studied.

REPORT ON POTASH

By L. D. HAIGH (Agricultural Experiment Station, Columbia, Mo.), *Associate Referee*

In studying the Fraps method for the determination of potash in mixed fertilizers as compared with the official method, the associate referee prepared two mixtures and submitted samples to collaborators.

Mixture No. 1 consisted of sodium nitrate, muriate of potash, dried blood and superphosphate compounded to make a 2–16–2 fertilizer. The theoretical percentages of potassium oxide present in the mixture, determined by potash determination on the separate constituents, amounted to 2.18 per cent.

Mixture No. 2 consisted of ammonium sulfate, muriate of potash, dried blood, superphosphate and a filler to make a 6–8–6 fertilizer. The theoretical percentage of potash present was 6.30 per cent.

The instructions sent to the collaborators included directions for the use of the Fraps method[2] as follows:

FRAPS METHOD FOR POTASH IN MIXED FERTILIZERS

Weigh 2.425 grams of the sample into a 250 cc. beaker; add 2 grams of precipitated calcium carbonate free from water-soluble potash and 75 cc. of water; mix well; and allow the mixture to stand in the cold for 3–4 hours, rotating the beaker occasionally. Transfer the contents of the beaker to a filter with hot water, receiving the filtrate into a 250 cc. graduated flask. Wash the material on the filter with successive portions of water, nearly boiling, until the volume of the washings is

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 48 (1931).
[2] *This Journal*, 9, 193 (1926).

200–250 cc. Cool to room temperature, make up to the mark, and draw off an aliquot into a porcelain dish. Place the dish on a water bath and evaporate to dryness; then add 1 cc. of strong nitric acid and 4 cc. of strong hydrochloric acid, and evaporate to dryness on a hot plate under a hood. Repeat the addition of nitric acid and hydrochloric acid and again evaporate to dryness. Then add strong hydrochloric acid and again evaporate to dryness to insure the removal of all nitric acid. Take up with hot water and add a few drops of hydrochloric acid and the usual quantity of platinum tetrachloride solution, using an excess sufficient to color the alcohol solution. Evaporate to a moist residue, not to complete dryness. Remove from the water bath and cover with 10 cc. of acidulated alcohol (10 cc. of strong hydrochloric acid to 100 cc. of 95 per cent alcohol). Allow to stand 1 hour, then filter into a Gooch crucible that has been thoroughly washed with hot water, transferring the insoluble residue to the crucible with the acidulated alcohol and washing until the filtrate is colorless. Wash the residue six to eight times with 10 cc. portions of the 20 per cent ammonium chloride solution and also with 80 per cent alcohol as in the official method, dry to 100°C. for 30 minutes, cool, and weigh. Remove the chloroplatinate from the Gooch by washing carefully with hot water, dry 2 hours, and weigh. For a 50 cc. aliquot, multiply the weight of the double salt by 40 to obtain the percentage of potassium oxide.

The associate referee is indebted to the following collaborators: P. McG. Shuey, Georgia; W. F. Hand, Mississippi; E. W. Magruder, Virginia; W. O. Collins, Georgia; J. H. Jolly, Louisiana; W. C. Geagley, Michigan; C. M. Bible, Pennsylvania. The results are given in the table in connection with the name of the laboratory chemist, when such name was furnished by the collaborator. A. R. Hall carried out the laboratory work at the Missouri Agricultural Experiment Station.

Results in percentage of K_2O.

	MIXTURE NO. 1		MIXTURE NO. 2	
	Official Method	Fraps Method	Official Method	Fraps Method
P. McG. Shuey	2.17	1.91	6.22	6.00
A. N. Lineweaver	2.31	2.07	6.09	6.15
M. P. Etheredge	2.15	2.04	6.18	5.93
W. O. Collins	2.22	2.15	6.23	6.09
J. H. Jolly	2.10	1.98	6.44	6.09
Percy O'Meara	2.27	2.16	6.61	6.60
C. M. Bible	2.16	2.10	6.23	6.48
A. R. Hall	2.17	2.15	6.20	6.37
Theoretical percentage of K_2O	2.18		6.30	

The results reported show quite uniformly lower results on Mixture No. 1 by the Fraps method. On Mixture No. 2 the results were also lower by the Fraps method in most cases. Exceptions to this were found by three collaborators, who obtained slightly higher results by the Fraps method.

It seemed probable to the associate referee that the collaborators would report higher results on Mixture No. 2 by the Fraps method than by the

official method. Mixture No. 2 contained ammonium sulfate, and the plan of the Fraps method is to avoid ignition of the solids by removing the ammonium salts by evaporation with nitric and hydrochloric acid. It seemed doubtful if all the ammonia could be volatilized from ammonium sulfate by nitric acid. The following experiment was tried to test out this point.

Four samples of ammonium sulfate were weighed out and analyzed for potash by the official method. Four more samples were likewise prepared and analyzed for potash by the Fraps method. Another set of aliquots from the Fraps method was handled in the same manner as the first four aliquots, with the exception that the residue from the nitric acid evaporations was heated long enough to volatilize any ammonium salts. The results of these tests were as follows:

	OFFICIAL METHOD		FRAPS METHOD		FRAPS METHOD WITH IGNITION	
		Average		Average		Average
Potash in ammonium sulfate	0.10		0.96		0.12	
	0.12		0.44		0.19	
	0.12	0.12	1.08	0.72	0 16	0.14
	0.15		0.41		0.08	

A fairly good agreement of results is shown by the two methods if the acid treatment in the Fraps method is followed by ignition, which suggests the possibility of an error being introduced into the potash determination when a large amount of ammonium sulfate is present in the mixture. Examples of such mixtures are the high analysis fertilizers of water-soluble potash and ammonium salts now being sold to the trade. Higher results on potash might be obtained on such mixtures if the Fraps method were used.

Most of the collaborators obtained no higher results on Mixture 2, containing ammonium sulfate, by the Fraps method than they did by the official method, which would seem to indicate that if complete volatilization of the ammonia is not effected by the nitric acid, the amount remaining is too small to influence the results. Probably the volatilization of the sulfate radical is assisted by the presence of some organic matter in solution.

Phosphates are not removed from solution by the Fraps method procedure, as given in this report, as was pointed out in a previous article,[1] in which a modification was suggested. However, this modification introduces calcium into solution, which is undesirable, as the Fraps method makes no provision for removal of calcium. If magnesium carbonate were used in place of calcium carbonate in the Fraps method, the writer's suggestion for complete removal of phosphate would be free from objection.

[1] *This Journal*, 10, 220 (1927).

C. M. Bible[1] suggested the removal of phosphates by means of magnesium oxide, but this suggestion has never been subjected to collaborative study by the association. As they are now conducted, phosphates are in solution in both the official and the Fraps method when the potash is precipitated as chloroplatinate.

It would seem from comments already made that phosphates are objectionable when ignition is practiced; in a method employing no ignition it is a question whether phosphates interfere.

RECOMMENDATIONS[2]

It is recommended—

(1) That the Fraps method be further studied, $MgCO_3$ being used in place of $CaCO_3$, and that the water-soluble phosphates be removed by the aid of heat.

(2) That the use of MgO in the official method, as suggested by Bible for the removal of water-soluble phosphates, be made a matter of study.

REPORT ON PLANTS

By O. B. WINTER (Agricultural Experiment Station, East Lansing, Mich.), *Referee*

Five of the six recommendations on plants approved by the association at its last meeting[3] were assigned to associate referees. The results of their work and the recommendations for the coming year will be given in their reports, which deserve the careful attention of the association.

The sixth recommendation, "That the methods for the determination of iron and aluminum be further studied and that more collaborative work be done on them," was to be carried out by the referee. The work on the determination of iron consumed much more time than was anticipated, and it was not completed in time for collaborative work. Hence no further study was made of the method for the determination of aluminum and no collaborative work was undertaken.

DETERMINATION OF IRON

During the past year, the claim has been made by Elvehjem[4] that the difficulties reported last year in the determination of iron are either directly or indirectly due to the presence of pyrophosphate, which is formed during the ashing of the sample.

The work on iron this year was directed toward trying to determine the effect of: (1) The acidity of the solution, (2) the presence of certain oxidizing agents, (3) the presence of phosphates, (4) the presence of pyro-

[1] *This Journal*, 8, 420 (1925).
[2] For report of Subcommittee A and action of the association, see *This Journal*, 14, 47 (1931).
[3] *This Journal*, 13, 220 (1930).
[4] *J. Biol. Chem.*, 86, 461 (1930).

phosphate, and (5) the quantity of potassium sulfocyanate present. The results of this work are not ready for publication, but they will be presented at an early date. The following conclusions, however, have been drawn: (1) The color intensity of the ferric sulfocyanate is dependent upon the acidity of the solution and upon the amount of sulfocyanate present, (2) a small amount of nitric acid prevents the reduction of the ferric salt, (3) with proper acidity and a sufficient excess of potassium sulfocyanate, the presence of phosphate causes no difficulty. The effect of the presence of pyrophosphate is still an open question. However, by boiling the solution for 30 minutes with the concentration of hydrochloric and nitric acids, as recommended in the technic for making the iron determination given in the next paragraph, any pyrophosphate present will be hydrolyzed to orthophosphate.

The optimum conditions for developing the ferric sulfocyanate color and the conditions that give consistent results in the determination of iron in the presence of phosphates and in which the fading is eliminated may be summed up in the following micro method for the determination of iron:

To an aliquot of the solution containing approximately 0.2 mg. of iron, add water to make about 40 cc., 5 cc. of concentrated hydrochloric acid and 0.3 cc. of concentrated nitric acid and boil for about 30 minutes. Transfer the mixture to a 50 cc. volumetric flask, add water to make about 35 cc., cool, add 10 cc. of 20 per cent potassium sulfocyanate solution, fill to the mark, and compare the intensity of color with that of a standard containing somewhere near the same amount of iron as the sample. Calculate the amount of iron present.

In order to learn whether the above method would be applicable for the determination of iron in a material high in phosphorus and extremely low in iron, a synthetic solution containing 0.180 mg. of iron and approximately two hundred times as much phosphorus as iron, was prepared and analyzed for iron. This solution also contained the other elements ordinarily found in a plant ash. Two materials known to have an extremely low iron content were also run by this method. The results of these analyses are found in Table 1.

TABLE 1.
Iron found in three samples.

SAMPLE NO 1	SAMPLE NO. 2	SAMPLE NO 3
mg.	*per cent*	*per cent*
0.182	0.00058	0.00071
	0.00061	0.00071
	0.00062	0.00068
	0.00057	0.00069
		0.00067

The results in Table 1 indicate that the method is applicable in the presence of an extremely small quantity of iron and a large quantity of phosphorus.

After making a careful review of the results obtained by the referees and the collaborators during the past several years on the study of the methods for the determination of iron and aluminum in plants, it was found that the macro methods now before the association are practically those that Patten (former referee on plants) submitted. These methods[1] have been modified and the results obtained on synthetic solutions agree well with the theoretical quantities of iron and aluminum present; the collaborative results also agree very closely. The results by the micro method for the determination of iron reported last year[2] were satisfactory for the materials analyzed. The difficulties encountered when running on materials high in phosphorus appear to have been eliminated by the method as modified in this report. The results by the micro method for the determination of aluminum[1] presented last year were also satisfactory. Therefore, in view of the fact that the methods now given in *Methods of Analysis* (1925) are obsolete, they should be replaced by these later methods, which are now being used almost universally.

During the last few years several requests have come to the Chemistry Department of this Experiment Station for fluorine determinations on feeds and animal excretions. Several attempts have been made at different times to determine the fluorine in these materials, but no reliable results were obtained. Furthermore, metabolism experiments in which fluorine is included in the feed are now being run on rats. Hence there is great need of a method for determining this element not only in the materials mentioned above, where an appreciable amount of fluorine may be present, but also a method for determining very small quantities such as may be present in plants and animal tisues. Since some other laboratories are also confronted with this problem, the chapter on plants should include a micro method for the determination of fluorine.

The referee has done considerable work on the above problem during the past year and suggests the following method as satisfactory for the preparation of the sample:

To from 1 to 50 grams of the finely ground material add about 0.01 gram of aluminum silicate for each gram of material taken and mix thoroughly. Saturate in a silica dish with a thin suspension of calcium hydrate and evaporate to dryness. Place in an electric muffle and completely ash below dull redness.

Either of the following two methods have been found promising for determining the fluorine in the ash: (1) the method described by Fairchild[3] in which the insoluble fluoride is fused with sodium carbonate, the sodium fluoride formed is separated from interfering elements and treated with a measured quantity of ferric chloride, and the excess of iron is determined iodimetrically; and (2) a combination and modification of the

[1] Patten and Winter, *This Journal*, 11, 203 (1928).
[2] *This Journal*, 13, 221 (1930).
[3] *J. Wash. Acad. Sci.*, 20, 141 (1930).

methods described by Deladrier,[1] Alimarin[2] and Casares.[3] This latter method consists in volatilizing the fluorine from the ash as silicon tetrafluoride by means of finely ground glass and sulfuric acid, collecting the silicon tetrafluoride in sodium hydroxide, acidifying very slightly with hydrochloric acid, and titrating with standard thorium nitrate, using a zirconium-alizarine mixture as the indicator.

In the analysis of plant materials, methods are needed for the determination of small quantities of calcium and phosphorus. The following microchemical methods, which have been used in the Laboratory of the Michigan Agricultural Experiment Station for a number of years, are giving satisfactory results. These methods should be incorporated in the 1930 revision of *Methods of Analysis*.

CALCIUM[4]

REAGENTS

(a) *Potassium permanganate.*—0.02 N.

(b) *Ammonium oxalate.*—Saturated solution.

(c) *Acetic acid (1+1).*—Mix equal volumes of glacial acetic acid and distilled water.

(d) *Ammonium hydroxide (1+1).*

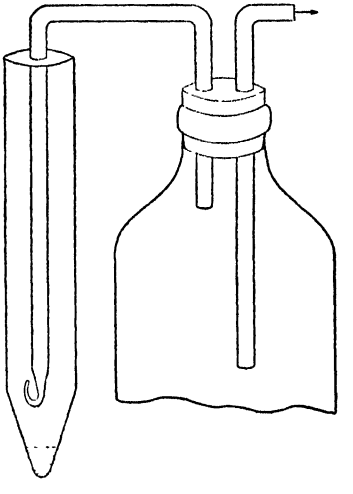
(e) *Ammonium hydroxide (1+49).*

(f) *Sulfuric acid (1+4).*

APPARATUS

(a) Conical-tipped centrifuge tubes about 15 cm. long and of about 18 mm. or 20 mm. inside diameter.

(b) Suction device with tip as shown in diagram.



(c) Centrifuge, about 2500. r.p.m.

DETERMINATION

Ignite 2 grams of the substance in a small Sillimanite crucible in a muffle at dull read heat. Dissolve the ash in hydrochloric acid (1+4) and transfer to a 100 cc. beaker. Add 5 cc. of strong hydrochloric acid and evaporate to dryness on the steam bath to dehydrate the silica. Moisten the residue with 5 cc. of strong hydrochloric acid, add about 50 cc. of distilled water, heat for a few minutes on the bath, transfer to a 100 cc. volumetric flask, cool quickly to room temperature, make to volume, shake, and filter, discarding the first portion of the filtrate. Pipet a 15 cc. aliquot into a conical-tipped centrifuge tube containing 2 cc. of saturated ammonium oxalate solution and 2 drops of 0.05 per cent methyl red. Add 2 cc. of dilute acetic acid, rotating the tube to thoroughly mix its contents. Add, while intermittently rotating the tube, ammonium hydroxide (1+4) until the solution is faintly alkaline, after which add a few drops of dilute acetic acid with a dropper until the color is adjusted to a faint pink (*p*H 5.0). (It is important at this point to rotate the tube so that the last bit of liquid in the coni-

[1] *Chem. Weekblad*, **1**, 324 (1903).
[2] *J. Anal. Chem.*, **81**, 8 (1930).
[3] *Ibid*, 66
[4] Shohl, *J. Biol. Chem.*, **50**, 527, 537 (1922); Kramer and Tisdall, *ibid*, **47**, 475 (1921).

cal tip is the color required.) Allow the mixture to stand at least 4 hours and whirl the tube in the centrifuge for 15 minutes. The precipitate should then be in a firm lump in the tip of the tube. Remove the supernatant liquid by means of the suction device shown in the diagram, taking care not to disturb the precipitate. Wash the precipitate by adding 2 cc. of 2 per cent ammonium hydroxide, rotating the tube to break up the precipitate. (It may be necessary to jar the tube sharply.) Return the tube to the centrifuge for 10 minutes, and again remove the supernatant liquid and wash with 2 per cent ammonium hydroxide as before. Repeat this operation until the precipitate has been washed three times. When the supernatant liquid has been removed after the final centrifuging add 2 cc. of sulfuric acid (1+4) to the tube, break up the precipitate as before, heat on the steam bath to between 80 and 90° and titrate in the tube with 0.02 *N* potassium permanganate, rotating the liquid during the titration to attain a proper end point. If the tube cools below 60°C. during the addition of the potassium permanganate, reheat it in the steam bath for a few minutes and complete the titration. Run a blank on an identical quantity of dilute sulfuric acid in a similar tube, heated to the same temperature, to determine the quantity of 0.02 *N* potassium permanganate necessary to give the color of the end point. Subtract this value from the buret reading. 1 cc. of 0.02 *N* potassium permanganate = 0.0004 gram of calcium. Report as percentage of calcium.

PHOSPHORUS[1]

REAGENTS

(a) *Potassium dihydrogen phosphate standard.*—Dissolve 0.4394 grams of pure dry KH_2PO_4 in distilled water and make up to a liter; 50 cc. of this solution, when diluted to 200 cc., gives a standard, of which 2 cc. = 0.05 mg. of phosphorus.

(b) *Ammonium molybdate.*—Dissolve 25 grams of ammonium molybdate in 300 cc. of water. Dilute 75 cc. of concentrated sulfuric acid to 200 cc. and add to the ammonium molybdate solution.

(c) *Hydroquinone.*—Dissolve 0.5 gram of hydroquinone in 100 cc. of distilled water, and add one drop of concentrated sulfuric acid to retard oxidation.

(d) *Sodium sulfite.*—Dissolve 200 grams of sodium sulfite in distilled water, make up to a liter, and filter. Keep this solution well stoppered or make it up fresh each time.

(e) *Magnesium nitrate.*—Dissolve 160 grams of magnesium oxide in nitric acid (1+1) avoiding an excess of the acid; add a little magnesium oxide in excess; boil; filter from the excess magnesium oxide, ferric oxide, etc.; and dilute to a liter.

PREPARATION OF SOLUTION

To 1 or 2 grams of the material in a small Sillimanite crucible add 1 cc. of magnesium nitrate solution and place on the steam bath. After a few minutes cautiously add a few drops of hydrochloric acid, being careful that the formation of gas bubbles does not push portions of the sample over the edge of the crucible. Make two or three further additions of a few drops of hydrochloric acid while the sample is on the bath so that as it approaches dryness there is a tendency for it to char. If the contents of the crucible become so viscous that no further drying may be obtained on the bath, complete the drying on a hot plate, put on a crucible cover, transfer to a cold muffle, and ignite at dull red heat for 6 hours, or until an even grey ash is obtained. It may be necessary to cool the crucible, dissolve the ash in a little water or alcoholic glycerol, evaporate to dryness, and return uncovered to the muffle for 4 or 5 hours longer. Cool, take up with dilute hydrochloric acid (1+4), and transfer to a 100 cc. beaker. Add 5 cc. of concentrated hydrochloric acid and evaporate to dryness on the steam bath to dehydrate the silica. Moisten the residue

[1] Briggs, *J. Biol. Chem.*, 59, 255 (1929).

with 2 cc. of strong hydrochloric acid, add about 50 cc. of distilled water, heat for a few minutes on the bath, transfer to a 100 cc. volumetric flask, cool immediately, make to volume, and filter, discarding the first portion of the filtrate.

DETERMINATION

To a 5 cc. aliquot of the filtrate in a 10 cc. volumetric flask add 1 cc. of ammonium molybdate, rotate the flask to mix, and allow to stand a few moments. Add 1 cc. of hydroquinone, again rotate the flask, and add 1 cc. of sodium sulfite. These last three additions may be made with a Mohr pipet. Make to volume with distilled water, stopper the mouth of the flask with the thumb or forefinger, and shake to thoroughly mix the contents. Allow to stand 30 minutes and compare immediately in a colorimeter with 2 cc. of the standard potassium dihydrogen phosphate solution treated simultaneously in an identical manner. With either the unknown or standard set at 25.0 mm., readings within 10 mm. (i.e., a range of 20 mm.) are accurate. If the concentration of phosphorus in the unknown is outside this range it may be brought nearer to that of the standard by diluting the filtrate, ashing a smaller or larger sample, making the filtrate to a smaller or larger volume, or using a smaller aliquot. Report as percentage of phosphorus.

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods for the determination of iron and aluminum in plants, as given in *This Journal*, 11, 203 (1928) and in *Methods of Analysis* (1925), p. 28, line 29, except that the fusion is made with a mixture of sodium and potassium carbonates instead of acid potassium sulfate, be adopted as tentative methods.

(2) That the micro method for the determination of iron presented in this report, and the micro method for the determination of aluminum given in last year's report (*This Journal*, 13, 221 (1930)) be adopted as tentative and that study be continued upon them with the object of making them official.

(3) That the microchemical method for the determination of calcium given in this report be adopted as tentative and that it be studied with the object of making it official.

(4) That the microchemical method for the determination of phosphorus given in this report be adopted as tentative and that it be studied with the object of making it official.

(5) That the methods referred to in this report and other methods for the determination of fluorine in plants be studied.

(6) That the reports of the associate referees be adopted.

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 48 (1931). For changes made for revision of *Methods of Analysis*, see *This Journal*, 14, 72 (1931).

REPORT ON PREPARATION OF PLANT MATERIAL FOR ANALYSIS

By H. R. KRAYBILL (Purdue University Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

At the 1929 meeting of the association the method submitted by the referee for preparation of sample for carbohydrates[1] was adopted tentatively.

Before this method is adopted as official it seems advisable to study the effect of time of storage of the sample on the various forms of carbohyhydrates. Preliminary studies have been made during the past year, but the results are not sufficient to recommend the final adoption of the method.

RECOMMENDATIONS[2]

It is recommended—

(1) That the effect of length of time of storage of the samples when preserved in alcohol on the various carbohydrates be studied by means of methods proposed by the associate referee.

(2) That methods of preparation of a sample for forms of nitrogen be studied.

REPORT ON LESS COMMON ELEMENTS IN PLANTS

By J. S. MCHARGUE (Department of Chemistry, Kentucky Agricultural Experiment Station, Lexington, Ky.), *Associate Referee*

In formulating a method for the determination of iodine in plant material, red clover was the plant material chosen for the following reasons: It is widely grown; it is easily collected and prepared for analysis; it can be readily ashed at a low temperature; and the plant apparently absorbs relatively large amounts of iodine from the soil.

METHOD

In a silica dish saturate 100 grams of the finely ground clover with a 10 per cent solution of iodine-free potassium carbonate, evaporate to dryness, ignite, and burn at as low a temperature as possible with a small Bunsen flame. After most of the organic matter has been consumed, transfer the dish to an electric furnace and completely ash below 400°C. Cool the dish and leach the ash with several small portions of hot water until the filtrate amounts to about 100 cc. Evaporate the filtrate to dryness; extract the residue with six portions of 5 cc. of 95 per cent ethyl alcohol, stirring until the residue assumes a pasty consistency; and evaporate the alcoholic extracts to dryness in a small beaker. Add 1 cc. of sulfurous acid, evaporate to dryness, and dissolve the residue in a few drops of hot water; filter into a 25 cc. separatory funnel; and wash, using only a few drops of water at a time until there is a volume of about 10 cc. in the funnel. Add 1 cc. of carbon disulfide, a few drops of

[1] *This Journal*, 13, 62 (1930).

[2] For report of Subcommittee A and action of the association, see *This Journal*, 14, 48 (1931).

dilute sulfuric acid, and about 1 cc. of a 10 per cent solution of sodium nitrite and shake vigorously for about 1 minute. If the carbon disulfide is colored pink, transfer a portion into a micro colorimeter cup and match with a known iodine standard prepared in the same way.

Results obtained for iodine on samples of clover grown on 8 plots at the Mayfield Experiment Field, Mayfield, Ky., are as follows:

PLOT TREATMENT	PARTS PER BILLION IODINE
No. 1—M	212
No. 2—ML	211
No. 3—MSP	308
No. 4—MRP	388
No. 5—MSPL	318
No. 6—MRPL	340
No. 7—MSPKL	332
No. 8—MRPKL	367
Average	310

It is recommended[1] that the study of the method for the determination of iodine in plants be continued.

REPORT ON TOTAL CHLORINE IN PLANTS

By MORTON F. MASON (Agricultural Experiment Station, East Lansing, Mich.), *Associate Referee*

Because of some difficulties in obtaining accurate results on certain materials with the proposed method given in the previous report[2] further study of the method itself seemed preferable to planning collaborative work. Determination of chlorine in pure organic compounds by this procedure was discontinued when it was found that there were many cases in which the method was entirely inapplicable. Since that time there has appeared an adequate method for determining halogen in such material.[3]

The method outlined in the 1929 report was unsatisfactory when it was necessary to digest a large amount of sample, 5–10 grams, in nitric acid containing silver nitrate in order to get an appreciable silver chloride precipitate. The determination is still questionable with some materials, but a few changes in procedure have increased its accuracy and range of application.

The modified method follows:

REAGENTS

(a) *0.02 N silver nitrate in concentrated nitric acid.*—Dissolve 3.3978 grams of pure silver nitrate in about 25 cc. of distilled water, transfer to a liter volumetric

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 49 (1931)
[2] *This Journal*, 13, 226 (1930).
[3] Thompson and Oakdale, *J. Am. Chem. Soc*, 52, 1195 (1930); Willard and Thompson, *ibid*, 52, 1893 (1930).

flask, and make to volume with nitric acid (sp. gr. 1.42). Transfer the mixture to a brown glass bottle to protect the solution from light.

(b) *0.05 N potassium thiocyanate.*—Dissolve 4.9 grams of potassium thiocyanate in distilled water, transfer to a liter volumetric flask, and make to volume. Standardize the solution against the 0.02 *N* silver nitrate, using ferric alum as an indicator.

DETERMINATION

Put a sample of 1–5 grams, containing not more than 10 mg. of chlorine, in a 250 cc. extraction flask containing 15 cc. of 0.02*N* silver nitrate in concentrated nitric acid, cover with a watch-glass, and allow to stand several hours or overnight. Heat on the steam bath, for 12–24 hours, until the digestion mixture is clear or nearly so, carefully rotating the flask at first so that the sample does not foam over. If necessary, add a little more nitric acid before the completion of the digestion. If there still remains some undigested residue associated with the small amount of silver chloride on the bottom of the flask, remove the flask from the bath and after a few minutes carefully add 0.5–1.0 gram of solid potassium permanganate. (The reaction may be quite violent.) Heat the flask gently with a Bunsen burner until there is no further reaction from the potassium permanganate. If the mixture immediately decolorizes, add permanganate until there is a slight excess, then decolorize by adding a few drops of glucose solution. Reheat the uncovered mixture on the bath until the volume is reduced to about 10 cc., add 5 cc. of glacial acetic acid, and boil 2 or 3 minutes. Cool, dilute with 10 cc. of distilled water, cool to the temperature of tap water, add about 0.3 of a gram of ferric alum, and titrate the excess silver with 0.05 *N* potassium thiocyanate. Run blank determinations on aliquots of a standard sodium chloride solution containing about 5 per cent sucrose to determine the corrections for chlorine in the reagents and the end point of the thiocyanate titration in the somewhat colored solution.

DISCUSSION

Allowing the sample to stand in the presence of cold nitric acid containing silver nitrate considerably reduces the foaming that results from immediate heating; in addition it cuts down the chances of loss of chlorine as free halogen during the initial violent action of the nitric acid and potassium permanganate upon the sample. Boiling with acetic acid, following the digestion, removes any cyanides that may be present in the solution. It also serves to lessen the often intense yellow-green color that makes detection of an end point with potassium sulfocyanate questionable. When the volume of the solution has been sufficiently reduced and acetic acid added, further dilution with water generally suffices to make an end point discernible. As a matter of fact, after a few trials the operator is often able to detect an appreciable end point when at first the color change seems to be most gradual. The rigorous treatment given the digestion mixture tends to deflocculate the silver chloride until it is a finely divided granular precipitate. With such an increase in surface exposed any error due to its slight solubility in potassium sulfocyanate becomes much larger; it may be kept at a minimum by speeding up the titration, hence it is advisable to run in nearly the required amount of potassium sulfocyanate from the buret and make the final approach drop by drop.

RECOMMENDATION[1]

It is recommended that work on total chlorine in plants be continued in view of the fact that the determination on some material still presents difficulty.

REPORT ON CARBOHYDRATES IN PLANTS

By J. T. SULLIVAN (Purdue University, Agricultural Experiment Station, Lafayette, Ind.), *Associate Referee*

A study was made of the more common carbohydrate constituents of plants: reducing sugars, sucrose, and starch. Because of the extreme complexity of plant composition, little of the abundant information that has been published concerning carbohydrate analysis can be applied unreservedly to any such material. Methods that are well known and in wide use are submitted for approval. Other methods, and those involving other forms of carbohydrates, cannot be offered without further study.

SUGARS

PREPARATION OF SOLUTION

Extraction.—Prepare the sample as described under "Preparation of Plant Material for Analysis, for Carbohydrates."[2] Pour the alcoholic solution through a filter paper or extraction thimble, catching the filtrate in a volumetric flask. Transfer the insoluble material to a beaker, cover with 80 per cent alcohol, warm on a steam bath for 1 hour, allow to cool, and again pour the alcoholic solution through the same filter. If the second filtrate is highly colored, repeat the extraction. Transfer the residue to the filter and allow to drain dry. Grind the residue so that all the particles will pass through a 1 mm. sieve, then transfer it to an extraction thimble, and extract for 12 hours in a Soxhlet apparatus with 80 per cent alcohol. Dry the residue and save for the starch determination. Combine the alcoholic filtrates and make to volume at a definite temperature with 80 per cent alcohol.

Clearing.—Place an aliquot of the alcoholic extract in a beaker on the steam bath and drive off the alcohol. Avoid evaporation to dryness by adding water if necessary. When the odor of alcohol has disappeared from the sample, add about 100 cc. of distilled water and heat to 80°C. to soften the gummy precipitates and break up insoluble masses. Cool to room temperature and proceed as directed under (*a*) or (*b*).

(*a*) Transfer the solution to a volumetric flask and rinse the beaker thoroughly with water, adding the rinsings to the contents of the flask. Add enough saturated neutral lead acetate to produce a flocculent precipitate, shake thoroughly, and allow to stand 15 minutes. Test the supernatant liquid with a few drops of saturated lead acetate. If more precipitate forms, shake, and allow to stand again. If no further precipitate forms, dilute to the mark with water, mix thoroughly, and filter through a dry filter. Add sufficient solid sodium oxalate to the filtrate to precipitate all the lead, and filter again through a dry paper. Test the filtrate for presence of lead with a little solid sodium oxalate.

(*b*) Add double the minimum amount of saturated neutral lead acetate solution that is required to cause complete precipitation, as found by testing a portion of the

[1] For report of Subcommittee A and action of the association, see *This Journal*, 14, 49 (1931).
[2] *This Journal*, 13, 62 (1930).

supernatant liquid with a few drops of dilute sodium oxalate solution. Without allowing the mixture to stand for more than a few minutes, filter immediately into a beaker to which has been added an estimated excess of sodium oxalate crystals. Allow the lead precipitate to drain on the filter and wash with cold water until the filtrate no longer gives a precipitate in the oxalate solution below. Excess of oxalate must be assured by testing with a drop of dilute lead acetate solution. Filter off and wash the precipitated lead oxalate, catching the filtrate and washings in a volumetric flask. Dilute to the mark with water and mix.

REDUCING SUGARS

Munson and Walker General Method

Proceed as in *Methods of Analysis, A. O. A. C.*, 1925, p. 190, **34 (a)** and **(b)** and **35.**

Quisumbing and Thomas Method[1]

REAGENTS

(a) *Copper sulfate solution.*—Wash crystals of C. P. copper sulfate free from dust, etc. with distilled water, dissolve in hot water to make a saturated solution, and filter. Determine the copper electrolytically and dilute the solution so that 25 cc. of it will contain 525 mg. of copper, or 41.2 grams of cupric sulfate pentahydrate in 500 cc. of solution.

(b) *Alkaline tartrate solution.*—Prepare a saturated solution of sodium hydroxide (purified by alcohol) and let stand for several days until the insoluble carbonates and other impurities have settled out. Siphon off the clear solution and establish its alkalinity by titration with standard acid. Dissolve 173 grams of highest purity Rochelle salts in water in a 500 cc. graduated flask and add the calculated amount of sodium hydroxide solution so that 500 cc. of this alkaline tartrate solution will contain exactly 65 grams of sodium hydroxide. Make to the mark with water.

PRECIPITATION OF CUPROUS OXIDE

Measure exactly 25 cc. each of the copper sulfate and alkaline tartrate solutions into a 400 cc. Pyrex or Bohemian glass beaker, the diameter of which is about 9 cm. Add 50 cc. of sugar solution containing preferably 50–150 mg. of sugar. Cover the beaker with a watch-glass and place in a water bath maintained at 80°C. After exactly 30 minutes, digestion, filter the cuprous oxide by suction through a mat of asbestos in a Gooch crucible. Wash the precipitate with warm water. Determine the copper by one of the methods below. Calculate weight of sugar from the tables of Quisumbing and Thomas.

REDUCED COPPER

I. Direct Weighing of Cuprous Oxide

Proceed according to *Methods of Analysis, A. O. A. C.*, 1925, p. 191, **36.**

II. Volumetric Permanganate Method

(a) Proceed according to *Methods of Analysis, A. O. A. C.*, p. 192, **39.**

(b) Filter and wash the cuprous oxide as directed above. Transfer the asbestos film to the beaker, suspend in water, and beat the precipitate and asbestos thoroughly. Rinse the crucible and the lip of the beaker with 10 cc. of a solution made by dissolving 240.9 grams of crystalline ferric ammonium sulfate and 200 cc. of concentrated sulfuric acid in water to 1 liter. Cool the diluted sulfuric acid before dis-

[1] *J. Am. Chem. Soc.*, **43**, 1503 (1921).

solving the alum. Receive the rinsings in the beaker containing the precipitate, washing the crucible and sides of the beaker with boiling water and catching the washings in the beaker; stir until all the copper is dissolved and a green solution is obtained. Titrate at once with continual stirring with potassium permanganate solution until the pink due to the permanganate persists for about 10–15 seconds. Deduct from the titration a blank, using distilled water instead of the sugar solution. One cc. of 0.05*N* potassium permanganate is equivalent to 0.0031785 gram of copper. Standardize the permanganate as follows: Dry overnight about 0.66 gram of sodium oxalate (pure) in a weighing tube in an oven at 100°C. and carefully weigh off three samples of 0.10–0.15 each. Dissolve each in 100 cc. of water, add 5 cc. of sulfuric acid (1+1), warm to 70°C., and titrate the permanganate against this. Take the average of the three titrations. One cc. of 0.05 *N* potassium permanganate is equivalent to 0.00335 gram of sodium oxalate.

III. Electrolytic Deposition from Sulfuric and Nitric Acid Solution

Proceed according to *Methods of Analysis, A. O. A. C.*, 1925, p. 192, **41.**

SUCROSE

(a) *Hydrochloric Acid Inversion.*—Proceed according to *Methods of Analysis, A. O. A. C.*, 1925, 119, **20.**

(b) When glucosides, which are easily hydrolyzed by hydrochloric acid, are present, sucrose may be inverted by invertase. The preparation and use of invertase is described in *Methods of Analysis, A.O.A.C.*, 1925, p. 183, **21.** The amount of invertase to be used depends on its activity. Avoid a large excess, which causes difficulty in the filtration of the reduced copper.

STARCH

Diastase Method with Subsequent Acid Hydrolysis

Proceed according to *Methods of Analysis, A. O. A. C.*, 1925, p. 119, **22,** and 120, **23** If the sample has been previously extracted in a Soxhlet with hot alcohol, further extraction with alcohol and ether is unnecessary.

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods presented be adopted as tentative for plants without further study.

(2) That studies be continued upon these methods with the object of adopting them as official or of modifying them.

(3) That studies be made especially upon methods of clearing, the determination of sucrose by the invertase method, and the determination of starch by the takadiastase method.

SELECTED REFERENCES

(1) Official and Tentative Methods of Analysis, A.O.A.C., 2nd ed. Washington, 1925.

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[1] For report of Subcommittee A and action of the association, see *This Journal*, **14**, 49 (1931).

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REPORT ON FORMS OF NITROGEN IN PLANTS

By HUBERT B. VICKERY (Connecticut Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

Although many methods have been proposed to determine the proportion of the water-soluble nitrogen of plant material that occurs in such simple forms as nitrate, ammonia, acid amides, etc., few of these methods can be applied to the special problem of the analysis of tobacco because of the presence in this plant of the volatile alkaloid nicotine. This substance is readily distilled with steam from alkaline solutions. As most methods for the determination of nitrates or amides depend upon the titration of ammonia isolated by distillation it is clear that artifices must be adopted when nicotine is present, whereby the results of the determination of ammonia shall not be vitiated.

Methods are described in this report for the determination of the proportion of the nitrogen in fresh or cured tobacco leaves that occurs in the form of nitrate and of ammonia. A method is also given for the determination of the proportion of the total nicotine of cured tobacco that is present in the so-called "free" form. Collaborative work on these methods is now in progress. A method has also been developed for the determination of amide nitrogen in tobacco, but inasmuch as this factor has no present significance in the ordinary examination of tobacco and the method is only applicable to protein-free extracts of the leaves, it need not be further referred to here.

NITRATE NITROGEN IN TOBACCO

This method is a modification of that described by Jones[1] for the analysis of fertilizers that contain such ingredients as cyanamide or urea. It is fully described by Vickery and Pucher.[2] The modification consists in the removal of the interfering nicotine before the reduction of the nitrate to ammonia; the relative magnitude of the blank is thereby greatly reduced and no special precautions are required during the distillation. The method follows:

[1] *Ind. Eng. Chem.*, 19, 269 (1927).
[2] *Ind. Eng. Chem., Anal. Ed.*, 1, 121 (1929).

REAGENTS

(a) *Concentrated sodium hydroxide solution.*[1]

(b) *0.1 N hydrochloric acid.*

(c) *0.1 N sodium hydroxide.*

(d) *Reduced iron powder.*—Determine the titration value of the ammonia in 3 ± 0.2 gram by distillation in the presence of sodium hydroxide.

(e) *Methyl red indicator.*[2]

DETERMINATION

Weigh two 2–5 gram samples of finely powdered dry tobacco into 800 cc. Kjeldahl flasks. (Three grams of reduced iron powder is not sufficient to care for the nitrate in 5 grams of tobacco that is exceptionally high in this constituent. If more than 0.5 per cent of nitrate nitrogen is found a 2 gram sample should be employed.) Add 30 cc. of water, a small piece of paraffin, a few angular quartz pebbles, and mix; add 5 cc. of concentrated sodium hydroxide solution and connect the flasks immediately to an apparatus arranged for distillation in a current of steam, the adapters of which are dipped beneath the surface of the fluid in the receivers. Collect the distillate from each apparatus in a flask that contains a suitable quantity (25-50 cc.) of 0.1 *N* hydrochloric acid. Admit steam to the flasks and heat with micro burners until the volume of liquid has been reduced to about 20 cc., then increase the supply of steam and adjust the burners so that this volume remains approximately constant. Distil until approximately 800 cc. has passed over (30–45 minutes). Reserve the distillate for titration (total volatile base) and for nicotine determination upon a suitable aliquot part.[3] Remove the flasks from the apparatus, wash down the steam inlet tubes and walls with 25 cc. of water, add 15–16 cc. of sulfuric acid (1+1) to each, and mix. Add 3 grams (± 0.2 gram) of reduced iron powder to one flask, rotate, and allow to stand until the reaction moderates. Place funnels in the necks of both flasks, heat them slowly, and finally boil the contents for 5 minutes with occasional shaking. Add 200 cc. of cold water, a few angular quartz pebbles, and 30–35 cc. of concentrated sodium hydroxide solution; connect at once to the Kjeldahl distillation apparatus; distil into a suitable quantity (10–35 cc.) of 0.1 *N* hydrochloric acid until 150 cc. has passed over; and titrate with 0.1 *N* sodium hydroxide. Add the titration value of the ammonia contained in the 3 grams of reduced iron powder to the titration value of the blank determination and subtract this from the titration value of the sample that has been reduced; the difference is the titration value of the nitrogen present as nitrate in the sample of tobacco taken.

AMMONIA IN TOBACCO

This method, an adaptation of Folin's technic for the determination of ammonia in urine, is based on the observation that nicotine does not undergo base exchange with permutit to a significant extent nor does it yield appreciable color with Nessler's reagent. A full description is given by Vickery and Pucher.[4]

REAGENTS

(a) *Ammonium sulfate stock solution.*—Dissolve 2.358 grams of pure salt in water and make up to 1000 cc.; 2 cc. = 1.0 mg. of nitrogen. Preserve by adding a few drops of chloroform.

[1] *Methods of Analysis, A.O.A.C.*, 1925, 7 (i).
[2] *Ibid.*, (k).
[3] *Methods of Analysis, A.O.A.C.*, 1925, 67.
[4] *J. Biol. Chem.*, 83, 1 (1929).

(b) *Ammonium sulfate standard solution.*—Dilute 200 cc. of (a) to 1000 cc.; 1 cc. = 0.1 mg. of nitrogen. Preserve with chloroform.

(c) *Nessler's solution* (Folin[1]).—Transfer 37.5 grams of potassium iodide and 27.5 grams of iodine to a 250 cc. flask, and add 25.0 cc. of water and 35–40 grams of mercury. Shake the flask continuously and vigorously for 7–15 minutes, or until nearly all the dissolved iodine has disappeared. (The solution becomes hot.) When the red iodine solution has begun to pale visibly, though still red, cool in running water, and continue the shaking until the reddish color of the iodine has been replaced by the greenish color of the double iodide. (This whole operation usually does not take more than 15 minutes.) Separate the solution from the surplus mercury by decantation and washing with liberal quantities of water. Dilute the solution and washings to 500 cc. If the cooling was begun in time, the resulting concentrated solution of the double iodide is clear enough for immediate dilution with 10 per cent sodium hydroxide and water and the finished Nessler's reagent can be used at once. Place 700 cc. of 10 per cent sodium hydroxide solution in a 1 liter flask, add 150 cc. of the clear concentrated solution of the double iodide, mix, and dilute to 1 liter with water. Allow to settle if a turbidity develops.[2]

(d) *Sodium hydroxide solution.*—10 per cent.

(e) *Permutit (Folin).*—Pass through sieves and reject material smaller than 80-mesh and larger than 60-mesh. Wash copiously with water by decantation until the whole settles rapidly and contributes no more dust or turbidity to the water. Dry in a current of air in a thin layer without heating. For recovery of permutit after use, see Folin's manual.[3]

DETERMINATION

Transfer an accurately weighed 0.5 gram sample of dry finely powdered tobacco to a 300 cc. Kjeldahl flask; add 25–30 cc. of water, then add a small piece of paraffin, a few angular quartz pebbles and 2–2.5 grams of light magnesium oxide. Prepare a stopper to fit the Kjeldahl flask with a piece of 9 mm. outside diameter glass tubing bent around through 180°, the short limb of the bend inserted through the stopper and the longer limb reaching to the level of the desk as the flask is held in a clamp over a micro burner. Greater convenience is obtained if the longer limb is cut and joined again by a short length of rubber tubing at a point about 15 cm. from the lower end. Connect the distillation tube so prepared to the flask and dip the lower end into a short wide test tube (50 cc. centrifuge tube) that contains 5 cc. of 0.1 *N* hydrochloric acid and a few drops of methyl red indicator. Heat the contents of the flask with a micro burner at such a rate that steam begins to rise from the receiver in about 3 minutes. Make no effort to cool the distillation tube nor the receiver. Distil for 5 minutes, counting the time from the point at which the distillate first runs down the tube; remove the tube and wash the end into the receiver with a few drops of water; cool the distillate; and dilute to 50 cc. Charge several 100 cc. volumetric flasks with 2.5–3.0 grams of washed and dried permutit and wash each several times by decantation with water. Transfer to three of the flasks 3 cc. of 0.1 *N* hydrochloric acid and 0.3, 0.5, and 1.0 mg., respectively, of ammonia nitrogen as standard ammonium sulfate solution. Add sufficient water to make each to a total volume of 25 cc. Transfer a 25 cc. aliquot of the distillate from each determination to a flask containing permutit. Shake all the flasks for 5 minutes with a gentle rotatory motion and lay them on their sides on a suitable support for 1 minute; decant the fluid from each flask and wash the permutit by decantation three

[1] Laboratory Manual of Biological Chemistry, New York, 4th ed., p. 293 (1926).
[2] This is Folin's method for the preparation of Nessler's reagent. The reagent prepared by the usual procedure is, however, equally satisfactory.
[3] Loc. cit.

times successively with 10–30 cc. of water, settling each time for 1 minute before decantation. Rinse the permutit to the bottom of each flask with 5 cc. of water, add 1 cc. of 10 per cent sodium hydroxide solution, and rotate for 3 minutes; add 65 cc. of water, rotate, and add 10 cc. of Nessler's reagent. Dilute to the mark, mix, and compare in a colorimeter the color of the solution derived from each determination with the known standard that most nearly matches it. The color is stable for several hours. Calculate the ammonia nitrogen as percentage of the sample of tobacco used.

"FREE NICOTINE" IN TOBACCO

A relationship has been noted between the reaction of extracts of tobacco as measured in terms of hydrogen-ion activity (*p*H) and the proportion of the nicotine which can be distilled with steam from the tobacco without the addition of alkali.[1] This proportion of the nicotine has been designated "free nicotine" by Garner,[2] and to it in part has been attributed the harsh and irritating effect experienced in the smoking of some tobaccos. The determination of "free nicotine" is therefore a matter of interest and possible importance in judging the quality of tobacco. The apparent dissociation constants of nicotine have been determined by Vickery and Pucher;[3] from these a curve can be constructed from which can be read the proportion of the nicotine that is in the free form at reactions between *p*H 5.0 and 9.0. Commercial tobaccos in general fall within these limits. The measurement is most conveniently carried out by means of a quinhydrone or hydrogen electrode and suitable potentiometer. Doubtless, however, one of the more refined colorimetric procedures could be employed with some sacrifice in accuracy.

DETERMINATION

Mix approximately 2.5 grams of dry powdered tobacco with 50 cc. of water. Stir for 5–10 minutes, allow to settle, and decant the necessary quantity into the cell of the quinhydrone or hydrogen electrode. Determine the *p*H value with an accuracy

FREE NICOTINE	*p*H	FREE NICOTINE	
per cent		*per cent*	*p*H
1	6.11	50	8.11
2	6.42	55	8.20
5	6.86	60	8.29
10	7.15	65	8.37
15	7.36	70	8.48
20	7.51	75	8.59
25	7.63	80	8.71
30	7.74	85	8.86
35	7.85	90	9.06
40	7.93	95	9.39
45	8.02		

[1] Conn. Agr. Expt. Sta. Bull. 295, p 338 (1928).
[2] U. S. Dept. Agr. Bur. Plant Ind. Bull. 141 (1909).
[3] *J. Biol. Chem.*, 84, 233 (1929).

of 0.1 unit. Construct a curve by plotting the data in the following table on a conveniently large scale. Read the percentage of free nicotine from this curve at a point corresponding to the *p*H found and report as percentage of the total nicotine in the free form.

RECOMMENDATIONS

It is recommended that collaborative study of the methods here outlined be continued during the coming year.

No report on dairy products was given by the referee.

REPORT ON MILK

By HENRY HOFFMANN, JR. (State Department Agriculture, Dairy and Food, St. Paul, Minn.), *Associate Referee*

As was recommended last year, the associate referee took up the proposed method for the determination of visible dirt in milk with the American Public Health Association and the American Dairy Science Association, because both of these associations are interested in this matter and recognize the need and value of a standard uniform method. Although the American Public Health Association has a method for this determination, there are many modifications of it in use. Various other methods are also used. Because there are so many methods in use and so many people are interested, it will require some time to work out a method satisfactory to all. It is hoped that those concerned with the use of this test will express their views. The associate referee will continue to work with the above-named organizations until a method satisfactory is obtained.

REPORT ON BUTTER

By C. W. HARRISON (U. S. Food and Drug Adm., Minneapolis, Minn.), *Associate Referee*

In 1925 Subcommittee C[1] recommended that an associate referee be appointed to study methods for the examination of butter, particularly methods for sampling and preparation of sample.

At the 1926 meeting, Mitchell presented a comprehensive report[2] of his studies of sampling and methods of analysis, including certain tentative recommendations for methods of sampling butter in tubs and prints. No mention was made, however, in either case of the number of individual packages which were to be sampled in any given lot, nor were the recommendations specific in regard to compositing the cores from individual units samples. The committee's request that year led to further work on

[1] *This Journal*, 9, 79 (1926).
[2] *Ibid.*, 10, 290 (1927).

sampling. The 1927 report[1] by Mitchell dealt largely with the composition of butter packed in tubs and in conclusion he repeated his recommendation for the adoption of the sampling methods given tentatively in his 1926 report. He also clarified somewhat the language of these former recommendations especially as regards the number of cores to be taken from a tub and leaving an option as to whether they should be taken from a single tub or a composite from three tubs in the same churn batch. He still failed, however, to give specific directions as to the number of packages to be sampled in a given lot.

This omission led Subcommittee C in its 1927 report[2] to make the following recommendations: "It is recommended—

"(1) That the method for sampling print butter given in the 1926 report and recommended by the associate referee for adoption as official be not adopted as official at this time, in order to give the referee an opportunity to conduct a study with a view to expanding the method to include directions as to the number of subdivisions necessary to represent a given lot of print butter. (2) That the method for sampling tub butter be further studied with the object of expanding this method to include the number of tubs to be sampled to represent a given lot of tub butter."

No report on butter was made by the associate referee in 1928 so Subcommittee C recommended[3] that work be continued as outlined the previous year. Practically the same action was taken in 1929.[4]

Because the associate referee considered that work on all six of the committee's recommendations was impossible in one year, he concluded to concentrate on recommendations (1) and (2), previously quoted, which deal with the subject of sampling butter packed in prints and tubs. The question of sampling a product like butter, which is not entirely homogeneous and varies somewhat in composition even within a given churn batch, presents a perplexing problem.

A sufficient number of sub-samples should be taken to represent as far as possible the composition of a given batch or shipment; at the same time the number of sub-samples must be held within bounds because it is obviously impractical to sample every tub or case of prints in a given lot.

The U. S. Food and Drug Administration recognized this fact in their sampling procedure in connection with the enforcement of the Federal Food and Drugs Act and adopted a definite schedule for the number of sub-samples to be withdrawn from any given lot. This sampling schedule covers both print and tub butter shipments and varies according to the number of individual tubs or cases in the lot and also as to whether the individual containers in the lot are designated by definite churn batch markings.

[1] *This Journal*, 11, 267 (1928).
[2] *Ibid.*, 75.
[3] *Ibid.*, 12, 77 (1929).
[4] *Ibid.*, 13, 242 (1930).

If churn markings are present, each churning is treated as a unit, and a sub-sample is taken to represent that batch. Samples from one churn batch are never composited with samples from a different churn batch.

The percentage number of sub-samples to be taken from any given shipment is based on the number of packages in the shipment, and it ranges from approximately 33 per cent in the case of small shipments to 10 per cent in the case of shipments of large size.

Owing to the close relationship that exists between the methods of analysis of this association and the enforcement of the Federal Food and Drugs Act,[1] it was decided to take the sampling procedure of the Food and Drug Administration as a guide in formulating a butter sampling procedure to recommend to this association. With this idea in view certain lots of butter at local dairies were sampled according to the schedule of the Food and Drug Administration and then this plan was extended to include a greater number of sub-samples from unit packages.

The sampling covered both tub and print butter and as far as possible was confined to sampling packages that were prepared from the same churnings. It was felt that this procedure would furnish more definite information than could be obtained by working on lots of butter consisting of a mixed lot of packages from different churnings and not identified by churn numbers.

In sampling tub butter the technic outlined by Mitchell was followed, one core being withdrawn with a trier from each of three tubs within the same churn batch and these three cores being composited in one container. A second composite sample was then taken from three additional tubs in the same churn batch. Altogether six churn batches from two creameries were sampled according to this procedure. The results of analysis are given in Table 1.

The results given in Table 1 show some variation in composition in the two composite samples taken from tubs in the same churn batch. This variation is no greater than might be expected, as it has been found that some individual difference in composition exists in practically every tub packed from the same churning.

This is illustrated in the results given in Table 2, where half of the tubs from one churning and all the tubs in another churning were sampled and analyzed individually.

While the results in Table 2 show that quite wide variation in composition may occur in some of the individual tubs of butter from a given churn batch, still the majority of the tubs do not show a wide variation from the average, so that a composite sample can be withdrawn which will fairly well represent the composition of the butter in all the tubs from a given churn batch. The work of the Food and Drug Administration in sampling butter in connection with regulatory operations has also shown

[1] Regulations for the Enforcement of the Federal Food and Drugs Act. S.R.A.F.D. No. 1, p. 4.

TABLE 1.

Composition of composite samples.

(One core from each of three tubs in churning)

DAIRY	CHURN NUMBER	TUBS IN CHURNING	MOISTURE	RESIDUE	FAT	FAT VARIATION
			per cent	*per cent*	*per cent*	*per cent*
N. C. Co.	(a)820	6	16.52	4.04	79.44	
	(b)	6	16.51	4.85	78.64	0.80
"	(a)827	16	16.13	3.70	80.17	
	(b)827	16	16.71	3.90	79.39	0 78
"	(a)835	13	15.96	3.80	80.24	
	(b)835	13	15.78	3.60	80.62	0.38
De S. Co.	(a)6	14	15.19	2.96	81.85	
	(b)6	14	15.33	3.07	81.60	0.25
"	(a)7	13	15.92	3.42	80.66	
	(b)7	13	15.50	3.39	81.11	0.45
"	(a)8	15	15.74	3.43	80.83	
	(b)8	15	15 75	3.35	80.90	0.07

TABLE 2.

Individual sampling of half and all the tubs in two churn batches.

DAIRY	CHURN NO.	MOISTURE	RESIDUE	FAT	SPREAD	
		per cent	*per cent*	*per cent*	*per cent*	
De S. Co.	5*	16.10	3.11	80.79	−0.10	
"	5	16.00	3.04	80.96	+0.07	
"	5	16.04	3.14	80.82	−0.07	
"	5	15.82	2.47	81.71	+0.82	Max. 81.71
"	5	15.93	3.63	80.44	−0.45	Min. 80.44
"	5	15.87	3.51	80.62	−0.27	Av. 80.89
"	9†	15.37	3.00	81.63	+0.14	
"	9	15.08	2.78	82.14	+0.65	
"	9	15.03	2.92	82.05	+0.56	
"	9	15.34	3.75	80.91	−0.58	
"	9	15.47	3.19	81.34	−0.15	
"	9	15.03	2.40	82.57	+1.08	
"	9	15.28	2.90	81.82	+0.33	
"	9	15.39	3.38	81.23	−0.26	
"	9	15.51	3.81	80.68	−0 81	
"	9	15.44	3.55	81.01	−0.48	
"	9	15.11	3.21	81.68	+0.19	
"	9	15.89	4.59	79.52	−1.97	
"	9	14.95	2.05	83.00	+1.51	Max. 83.00
"	9	15.32	3.22	81.46	−0.03	Min. 79.52
"	9	15.62	3.04	81.34	−0.15	Av. 81.49

* Churn batch consisted of 13 tubs.
† Churn batch consisted of 15 tubs.

this to be a fact. Innumerable shipments from which preliminary samples were taken at the point of origin and found on analysis to be deficient in fat, when resampled at destination almost always confirmed the original findings, and the results of analysis on the two samplings were in remarkably close agreement.

While the sampling of tub butter presents a rather difficult problem, the sampling of print butter presents even greater difficulties. The reason for this is three fold: *First*, butter packed in tubs is generally identified by numbers indicating the churn batch, so that from a given shipment it is usually possible to pick out the number of churn batches involved and sample each batch individually. As butter in prints is generally not so identified, it is impossible in most cases to know how many churn batches are involved and therefore to know the relationship of one case of prints to the next.

Second, the tub being a larger unit package, can be sampled with a trier with a fair chance of withdrawing a core representative of the composition of the whole tub, while in the case of the individual print, one taken from a case may or may not be fairly representative of the composition of the rest of the prints in the case.

Third, in the case of tubs it is possible with the trier to withdraw cores from several tubs in the same churn batch and composite these into one sample and such a sample should be more representative than a core from only one tub. In sampling prints each print taken must be considered as an individual sub-sample, and no compositing is possible because the relationship of one print to another is not known.

Here, again, it is necessary to apply a rule of reason and in sampling any given lot of print butter to withdraw a sufficient number of subsamples to be representative of the lot as a whole, recognizing the fact that the number to be taken must not be so great as to overburden the laboratory or to make the cost of samples prohibitive. The sampling procedure used by the Food and Drug Administration in regulatory operations was used as a guide.

The sampling procedure involves taking a high percentage of subsamples from a small shipment with gradually decreasing percentage of sub-samples as the number of packages in the shipment increases.

Butter in prints is usually shipped packed in cases whose capacity is 30 pounds. The individual prints may be wrapped only in waxed papers or parchment, but more usually these wrapped prints are also enclosed in a carton which is also generally waxed so as to be waterproof. The individual units in the carton may be in pound, half-pound or quarter-pound size. In general a sample should consist of not less than one-half pound.

With a view to determining the probable number of individual prints which it would be necessary to collect from a given shipment to be representative of the lot, certain lots were selected which contained not more

than 10, and between 10 and 25, 30 pound cases, respectively. All the cases in each individual lot sampled were believed to contain prints prepared from the same churn batch.

From each of the ten case lots, one individual print was drawn from each of three cases, then two additional prints from each of two additional cases. In other words, this sampling represented 33 per cent and 50 per cent, respectively, of the cases. Each of these individual prints was placed in a separate container and analyzed as a separate sub-sample.

With lots containing more than 10 and not more than 25 cases, 5 individual prints were taken, one from each of 5 cases, and in addition two extra prints, one from each of two additional cases. This represented an approximate sampling of 20 and 30 per cent of the cases in the lot. It was hoped that this multiple sampling might furnish valuable information relative to the variation in composition with might be expected to occur in the individual prints taken from various cases in a lot and all of which were believed to have been prepared from the same churn batch.

The results of analysis are presented in Tables 3 and 4.

The results given in Tables 3 and 4 show that there may be considerable variation in composition of individual prints of butter even when they originate from the same churn batch.

TABLE 3.

Composition of individual prints taken from small (10 or less cases) churn batch.

DAIRY	CASES IN LOT	CHURN BATCH NO.	MOISTURE	RESIDUE	FAT	
			per cent	*per cent*	*per cent*	
De S. Co.	10	227	15.17	3.09	81.74	
"	10	227	15.21	2.70	82.09	Av. of 3 =
"	10	227	14.84	2.83	82.33	82.05
"	10	227	15.17	2.78	82.05	Av. of 5 =
"	10	227	15.37	2.62	82.01	82.04
N. C. Co.	10	925	15.78	3.24	80.98	
"	10	925	16.01	4.13	79.86	Av. of 3 =
"	10	925	16.33	4.50	79.17	80.00
"	10	925	15.67	3.50	80.93	Av. of 5 =
"	10	925	16.00	4.27	79.73	80.11
M. C. Co.	10	9204	15.73	3.90	80.37	
"	10	9204	15.87	3.73	80.40	Av. of 3 =
"	10	9204	15.84	3.56	80.60	80.46
"	10	9204	15.67	3.64	80.69	Av. of 5 =
"	10	9204	15.96	3.49	80.55	80.52

TABLE 4.

Composition of individual prints taken from large (10 to 25 cases) churn batch.

DAIRY	CASES IN LOT	CHURN BATCH NO.	MOISTURE	RESIDUE	FAT	
			per cent	*per cent*	*per cent*	
De S. Co.	25	242	14.73	3.41	81.86	
"	25	242	14.95	3.22	81.83	
"	25	242	14.94	3.09	81.97	
"	25	242	13.22	2.78	84.00	Av. of 5 =
"	25	242	13.51	2.80	83.69	82.67
"	25	242	15.02	3.40	81.58	Av. of 7 =
"	25	242	15.93	3.39	80.68	82.23
N. C. Co.	24	934	15.38	3.34	81.28	
"	24	934	14.66	3.22	82.12	
"	24	934	14.76	3.23	82.01	
"	24	934	14.96	3.14	81.90	Av. of 5 =
"	24	934	14.73	3.33	81.94	81.85
"	24	934	14.98	3.15	81.87	Av. of 7 =
"	24	934	15.06	3.54	81.40	81.79
M. C. Co.	23	9203	15.92	3.44	80.64	
"	23	9203	15.93	3.37	80.70	
"	23	9203	15.72	3.37	80.91	
"	23	9203	15.91	3.49	80.60	Av. of 5 =
"	23	9203	16.29	3.56	80.15	80.60
"	23	9203	15.91	3.49	80.60	Av. of 7 =
"	23	9203	15.86	3.45	80.69	80.61

The average butter fat content found on sampling increased beyond what may be considered a reasonable sampling of a given lot and does not differ materially from the average fat content found in the smaller number of sub-samples. It is therefore felt that if sub-samples be withdrawn from a fair percentage of cases in a lot of print butter the average fat content of such a sampling will be fairly representative of the lot of butter as a whole.

Using the results reported in the tables as a basis, and the experience gained in sampling shipments of butter during the course of regulatory work, and recognizing the fact that the number of sub-samples that can be withdrawn from any given lot must be limited by certain practical considerations, the associate referee presents the following procedure for sampling butter in tub and print forms.

TUB BUTTER

Always sample according to churn batch numbers if these are available, treating each batch as an entity and in no case composite cores taken from

packages bearing one batch number with those obtained from another churn batch.

Withdraw the sample with an ordinary type butter trier, either warm or cold depending on the physical state of the butter, and take the core by inserting the trier vertically through the butter mass at a point approximately half-way between the center and the edge of the tub. If more than one core is taken from a tub, withdraw them at different points around the circumference of an imaginary circle half-way between the center and side of the tub. Refill all trier holes with a plug of butter approximately one inch long, which may be procured by withdrawing an extra core and cutting it into lengths.

The following schedule may be considered a minimum sampling; it may be increased if circumstances indicate the necessity for a greater number of sub-samples.

(a) In the case of tubs marked with churn batch numbers, take from each batch one composite sample consisting of three full cores taken with a trier, one from each of three tubs in the batch.

(b) If tubs are not marked with churn batch numbers, sample the following number of tubs in the lot:

Number of tubs	Sample not less than—
1– 10	3
11– 25	5
26– 50	6
51– 75	8
76–100	10

Each sub-sample should consist of three cores taken from one tub with the trier and placed immediately in the sample container.

Butter stored at a moderate temperature can best be sampled with a trier which has not been warmed; very hard butter which has been held at a fairly low temperature is best sampled with a trier which has been warmed; frozen butter from a sharp freezer or that has been exposed to subzero temperatures cannot be sampled with a trier, but must first be placed in a tempering room for 24 hours before sampling.

The core is obtained by inserting the trier for practically its full length and giving it one complete turn, thus withdrawing a full core. Transfer the core directly to the sample container with the aid of a spatula. Do not include moisture adhering to the outside of the trier in the sample.

After the core is removed from the trier the instrument should be wiped clean and dry before taking the next core.

PRINT BUTTER

The word "print" used in this report refers to the contents contained in the unit package or carton, either the one pound or the wrapped half or

quarter pound prints if these units are contained in the carton. Each sub-sample should consist of at least one-half pound.

In sampling print butter treat each sub-sample as a unit and do not composite the sample by including portions of units from different prints in one container.

The following is suggested as the minimum number of sub-samples to be collected in sampling a given lot of print butter. This schedule is based on the assumption that the individual prints are packed in shipping cases. The usual commercial practice is to pack thirty prints (pounds) in each shipping case.

Cases	Sub-samples	1 from each of—cases
1– 10	3	3
11– 25	5	5
26– 50	6	6
51– 75	8	8
76–100	10	10

If any considerable delay is to occur between the time of collecting the sample and the analysis, which might result in loss of moisture, the wrapping should be removed from the print and the butter transferred to the sample container to prevent any possible loss of moisture.

SAMPLE CONTAINERS

The question of proper containers for butter samples has been agitated from time to time and in 1926 Subcommittee C recommended that the suitability of metal containers be tested.

The associate referee in 1929 gave certain fundamental objections to the use of metal (tin) containers for butter samples and stated a preference for glass. The present referee agrees with the conclusions reached by the previous referee in this matter. It is believed that it is highly desirable that the sample be contained in a transparent container so that the analyst may observe it during mixing. Also if any leakage of water into the container occurs he can observe it before the sample is melted and mixed. Further, the tops of metal containers are not apt to be absolutely water-tight, especially after use once or twice and water may leak into the sample during storage or melting.

The best type of container for butter samples is believed to be the glass jar fitted with new rubber rings which when equipped with the proper type of top will prevent the possibility of loss of moisture through evaporation or the entrance of water through leakage. The Mason jars with glass tops or jars provided with the "Kerr self sealing cap are practical and meet the specifications." Mason jars with metal tops are believed inferior to the glass top variety on account of possible loss of water from the sample between the cap and the screw threads on the jar.

RECOMMENDATIONS[1]

It is recommended—

(1) (a) That print butter be sampled according to the procedure detailed in this report, the sampling schedule given being considered as a minimum of the number of sub-samples to be withdrawn when sampling any given lot.

(b) That while print butter is rarely designated by churn batch numbers, if such numbers are present each batch be sampled individually, as directed in sampling tub butter identified by batch numbers, with the exception that the three sub-samples withdrawn be not composited but analyzed separately.

(2) That tub butter be sampled according to the procedure detailed in this report, with the same limitation as suggested in the case of butter in prints.

(3) That recommendations (4) and (5) of Subcommittee C's report[1] be carried over for further study by the associate referee next year.

(4) That the glass jar, preferably with a glass top, be approved as the proper receptacle to contain butter samples and that the container should be of a type which will prevent loss of moisture by evaporation or leakage of water into the jar (first action).

(5) That the proposed mechanical stirrer method given in the 1927 report[3] be adopted as a tentative method.

No report on cheese was made by the associate referee.

REPORT ON DRIED MILK

By E. L. P. TREUTHARDT (Food and Drug Administration, Boston, Mass.), *Associate Referee*

The work of the previous year[4] showed that the tentative method for the determination of fat in dried milk, as modified, was not sufficiently accurate. In lieu of collaborative work this year, the associate referee studied certain details which might assist in obtaining more concordant results. This work was done on whole milk powders, because they present the most difficulty and also constitute an important problem in regulatory work.

The recommendation made last year to study the sampling of dried milks suggests that a non-homogeneous condition may be a source of er-

[1] For report of Subcommittee C and action of the association, see *This Journal*, **14**, 55 (1931).
[3] *This Journal*, **11**, 75 (1928).
[3] *Ibid.*, 267.
[4] *Ibid.*, **13**, 245 (1930).

ror. The tentative method for preparation of sample[1] was devised for malted milk, and therefore it does not provide for the condition frequently met in dried whole milk. W. S. Hubbard of the Schwarz Laboratories, Inc., New York, suggested sifting to prepare the sample for analysis. A 20-mesh sieve was found to be the most practical for whole milk powder. The large soft lumps could be broken up, and by rubbing the material and tapping the sieve vigorously all but a small residue was passed through. This residue consisted mainly of hard granular particles, evidently produced by fusing, which could be worked through the sieve and mixed with the sifted portion after they had been ground in a mortar. Some samples contained small pieces of wood, and one sample contained a piece of metal. A small loss usually occurred during sifting when particles were blown away and adhered to the sieve.

The results obtained by P. L. Leavitt on sifting eight samples through a 20-mesh sieve are given in Table 1; they show the possibility of error in using an unsifted sample.

TABLE 1.

Separations in sifting dried milk samples.

SAMPLE NO.	SUBSTANCE	WEIGHT OF SAMPLE	GRANULAR RESIDUE	WOOD PARTICLES	LOSS
		grams	*per cent*	*per cent*	*per cent*
1	Skim milk	515	0	0	0.70
2	Previously sifted whole milk	107	0	0	0
3	Whole milk	153	0.09	0.01	0.15
4	Whole milk	293	0.78	0	0.28
5	Whole milk	123	0.57	0	0.65
6	Whole milk	306	0.13	0.16*	0.20
7	Whole milk	369	0.06	0.02	0
8	Whole milk	105	0.08	0.01	0.67

* Wood and metallic particles.

To study the effect of temperature and time of heating, two series of determinations were made by the associate referee, who used the tentative method for fat. The sample was sifted three times, and all coarse material was discarded. The charges were heated in a water bath at 60°C. or on top of a steam bath for periods varying from 3 to 30 minutes. The portions for each series were weighed out at one time. Moisture determinations made on portions taken at the start and finish of the weighings showed no absorption of moisture by the sample during the time required to weigh out twelve portions.

The results are given in Table 2.

[1] *This Journal*, 10, 35 (1927); *Methods of Analysis, A.O.A.C.*, 1925, 275.

TABLE 2.

Fat in dried milk—effect of temperature and time of heating.

	PLACE OF HEATING	TEMPERATURE	TIME	FAT (purified)	IMPURITY IN FAT
		°C.	*Minutes*	*per cent*	*per cent*
1st series	Steam bath	67	3	23.20	0
				23.09	0
	Steam bath	72–76	10	23.32	0
				23.38	0.06
	Water bath	60	10	23.58	0.04
				24.53	0
	Water bath	60	30	23.42	0.15
				23.43	0
2nd series	Water bath	60	3	23.53	0.08
				23.51	0
	Steam bath	74	3	23.36	0
				23.54	0
	Water bath	60	10	23.24	0
				23.56	0
	Steam bath	67	10	23.34	0.05
				23.15	0.06
	Water bath	60	30	23.21	0 05
				23.62	0.33
	Steam bath	67	30	23.09	0
				23 49	0.12

According to the results given in Table 2, considerable variation is permissible in heating, and the wording of the tentative method "warm on the steam bath" appears sufficient.

A single small glass bead placed in the tared flask prevented violent ebullition of ethers on the steam bath. This bead was weighed with the flask and remained in it when the fat was finally dissolved in petroleum ether.

A non-fat residue in about half the determinations, occasionally of significant magnitude, confirms the necessity for purification of the fat in all cases. Accordingly, weighing of the empty flask at the start appears unnecessary. The flask may be weighed first after evaporation of the solvent and drying of the fat. After reaching constant weight the fat is removed with three portions of petroleum ether, and the flask with the residual im-

purities is heated, cooled, and reweighed. This procedure is of advantage in that both weighings can readily be made within a short time with small error due to variations in temperature and humidity of the laboratory. Care should be taken to weigh the flask as it comes from the desiccator, that is without wiping, as considerable error results from weighing a recently-wiped flask.

Further study of the method for fat should give attention to the practicability of weighing the sample directly into the extraction apparatus, to variation in the quantities of ammonia and alcohol, to the size of the weighing flask, and to the substitution of a weighing dish for the flask.

The American Dry Milk Institute determines moisture by distillation in a Bidwell-Sterling apparatus.[1] It is believed that this method should be tested by the association.

RECOMMENDATIONS[2]

It is recommended—

(1) That in place of the malted milk method, the following method for preparation of sample of dried milk be adopted as tentative and be given further study:

Sift the sample through a 20-mesh sieve onto a large sheet of paper, rubbing the material through the sieve and tapping vigorously if necessary. Grind the residue in a mortar, pass through the sieve, and mix into the sifted material. Particles of wood and of other material that cannot be ground may be discarded. Sift the sample twice more, mixing thoroughly each time. To avoid absorption of moisture, operate as rapidly as possible and preserve the sample in an air-tight container.

(2) That further study be made of the details of the tentative method for determination of fat in dried milk, especially as regards weighing the sample directly into the extraction apparatus and the quantities of reagents used.

(3) That the official Roese-Gottlieb method for fat[3] be amended by omitting the first weighing of the flask and requiring the final extraction of fat in petroleum ether, by requiring the use of a bead in the flask, and by inserting a warning against wiping the flask just before weighing.

(4) That study be made of the sampling of dried milks.

(5) That study be made of the distillation method for determining moisture in dried milk.

[1] *J. Ind. Eng. Chem.*, 17, 147 (1925); *This Journal* 8, 2,95 (1925).
[2] For report of Subcommittee C and action of the association, see *This Journal*, 14, 56, 79 (1931).
[3] *Methods of Analysis, A.O.A.C.*, 1925, 262.

REPORT ON MALTED MILK

By F. HILLIG (U. S. Food and Drug Adm., Washington, D. C.),
Associate Referee

Complying with last year's recommendation on malted milk, which provides for the study of its identification microscopically,[1] samples were submitted for collaborative work. One sample consisted of genuine malted milk, and the other three samples were mechanical mixtures, the compositions of which are indicated in the following tabulation of results obtained by the collaborators.

SAMPLE CONTAINED	COLLABORATIVE RESULTS				
	1	2	3	4	5
A Malted milk	Correct	Correct	Correct	Correct	Correct
B Malted milk (50%) Sugar (35%) Cocoa (15%)	Correct	Correct	Correct	Sugar not reported	Reported malt extract and dried milk instead of malted milk
C Spray dried malt extract (50%) Spray dried whole milk (50%)	Correct	Correct	Correct	Correct	Correct
D Spray dried malt extract (10%) Spray dried whole milk (10%) Sugar (65%) Cocoa (15%)	Correct	Skim milk reported instead of whole milk	Correct	Correct	Skim milk reported instead of whole milk

Only one collaborator (No. 5) failed to recognize malted milk in admixture with sugar and cocoa (Sample B). For Sample D all collaborators agreed upon spray-dried malt extract, cocoa, and sugar; however, two of the collaborators experienced trouble in identifying whole milk. From the results shown it is evident that malted milk is easily distinguished microscopically from other materials of similar composition.

No comments or suggestions were reported by the collaborators.

Unfortunately, time did not permit of further study of the determination of butterfat, as recommended.

[1] *This Journal*, 12, 238 (1929).

RECOMMENDATIONS[1]

It is recommended—

(1) That the microscopical procedure for the identification of malted milk be adopted tentatively, and that no further study on the subject be made.

(2) That methods for the determination of butterfat in malted milk be further studied.

No report on ice cream was given by the associate referee.

REPORT ON MILK PROTEINS

By HENRY C. WATERMAN (Office of Experiment Stations, Washington, D. C.), *Associate Referee*

In last year's report on a method for the approximate isoelectric precipitation of casein from milk,[2] a number of suggestions from collaborators regarding a procedure for hastening a slow filtration were noted. Further work on the method, modified to obtain a clear filtrate by direct filtration, was recommended and authorized. This work has been done by two collaborators. J. T. Keister obtained figures a very little higher than those obtained by the use of Official Method I for casein. Filtration was conducted about as before and was very slow. Hartmann and Hillig made up the mixture of sample and precipitant to volume as directed, and then added 0.5 gram of filter-cel, shook thoroughly, and filtered at once, using a C. S. and S. folded paper No. 588. Filtration was completed in about 10 minutes, and the filtrate was clear. They reported that filtration as originally directed was so slow that the work was not finished.

RECOMMENDATIONS[3]

It is recommended—

(1) That the filtering direction of the method as modified last year be replaced by the words: "add 0.5 gram of filter-cel, shake thoroughly, and filter clear through a suitably folded filter paper."

(2) That the method thus modified be adopted as tentative.

(3) That the method, modified as here recommended, be given further collaborative trial with a view to its adoption as official, first action, next year.

No report on qualitative tests was given by the associate referee.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 55 (1931).
[2] *This Journal*, 10, 259 (1927).
[3] For report of Subcommittee C and action of the association, see *This Journal*, 14, 56 (1931).

REPORT ON FATS AND OILS

By GEORGE S. JAMIESON (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

During the past year collaborative study has been made on the determination of moisture and volatile matter by three procedures known as the hot plate, air oven, and vacuum oven methods. The directions for this determination by the three methods are as follows:

MOISTURE AND VOLATILE MATTER

Method No. 1—Vacuum Oven Method

APPARATUS

F.A.C. standard vacuum oven.[1]—Or an equivalent vacuum oven.

Moisture dish.—A shallow glass dish, lipped, beaker form, approximately 6–7 cm. diameter and 4 cm. deep, shall be standard.

DETERMINATION

Soften the sample if necessary by means of gentle heat, taking care not to melt it. When sufficiently softened, mix thoroughly with a mechanical egg beater or other equally effective mechanical mixer.

Weigh 5 grams (±0.2 gram) of the prepared sample into a moisture dish. Dry to constant weight in vacuo at a uniform temperature not less than 20° nor more than 25° above the boiling point of water at the working pressure, which shall not exceed 100 mm. of mercury. Constant weight is attained when successive dryings for 1-hour periods show an additional loss of not more than 0.05 per cent. Cool the sample in an efficient desiccator (one-half hour) and reweigh. Report the percentage loss in weight as moisture and volatile matter.

The boiling points of water at reduced pressures are as follows:

Pressure	°C	Temperature of Oven	
mm.		*mm.*	*maximum*
100	52	72	77
90	50	70	75
80	47	67	72
70	45	65	70
60	42	62	67
50	38	58	63
40	34	54	59

Method 2.—Hot Plate Method[1]

DETERMINATION

Weigh out 5–20 gram portions of the prepared sample (see Method I) into a glass beaker or casserole and heat on a heavy asbestos board over a burner or hot plate, taking care that the temperature of the sample does not at any time go above 130°C. During the heating rotate the vessel gently by hand to avoid sputtering or too rapid evolution of moisture. Judge the approach of the end point by the absence of rising bubbles of steam and by the absence of foam at the last, but continue the heating momentarily to incipient smoking (caution). Cool in a desiccator one-half hour and weigh.

[1] *Ind. Eng Chem.*, 18, 1350 (1926).

Limitations.—This method is applicable to all the ordinary fats and oils, including emulsions such as butter and oleomargarine and high-acid coconut oil, but it is not applicable to certain abnormal samples, such as naphtha extraction greases which contain, in addition to moisture, solvents of fairly high boiling point that are driven off with difficulty. In handling such samples, it is possible to obtain satisfactory results by using the Kingman distillation method for actual moisture and steam distillation for the solvents. In difficult cases it may be advisable to determine the actual saponifiable matter present.

Method 3.—Air Oven Method

APPARATUS

Air oven.—A well-constructed, well-ventilated oven held uniformly at a temperature of 105°–110°C. Keep the thermometer bulb close to the sample. For drying and semi-drying oils, keep an atmosphere of carbon dioxide or some other inert gas in the oven.

Moisture dish.—A shallow glass dish, approximately 6–7 cm. diameter and 4 cm. deep.

DETERMINATION

Weigh 5 grams (±0.2 gram) of the prepared sample (See Method I) into a moisture dish. Dry to constant weight. (Constant weight is attained when successive dryings for 1-hour periods show an additional loss of not more than 0.05 per cent.) Cool the sample in an efficient desiccator (one-half hour) and reweigh. Report the percentage loss in weight as moisture and volatile matter.

The samples of oils submitted to the collaborators were as follows: No. 1, crude corn oil; No. 2, crude cottonseed oil (from South Carolina); No. 3, crude menhaden oil; and No. 4, crude cottonseed oil (from Tennessee). All the samples were commercial oils which varied in size from 1 to 5 gallons.

The following collaborators reported results: (1) D. A. Edeler, (2) F. Fenger, (3) J. T. Keister, (4) R. S. McKinney, (5) W. D. Richardson, reporting for Analysts 1 and 2, (6) M. L. Sheeley, (7) F. W. Kirk.

An examination of the reports from the collaborators indicates that the hot plate method gives higher results in many instances than either of the two other methods studied. The closest agreement among the collaborators was obtained with the vacuum oven method. With one exception, unfortunately, the samples submitted for study were low in moisture, but it it is believed that some useful information has been obtained from this work. From the wide variations in the results obtained by the hot plate method with sample 2, which had the highest content of moisture and volatile matter, it appears evident that further work should be done towards standardizing this procedure, particularly with samples high in moisture. A satisfactory comparison of the results obtained by the three methods is impossible owing to the low moisture content of three of the

samples. A constant weight was assumed (as directed) when the loss of the sample after heating did not amount to more than 0.05 per cent.

Percentages of moisture and volatile matter by three methods.

ANALYST	HOT PLATE A	HOT PLATE B	AIR OVEN A	AIR OVEN B	VACUUM OVEN A	VACUUM OVEN B
Sample 1						
1	0.10	0.08	0.04	0.07	0.04	0.03
2	0.12	0.13	0.06	0.06	0.10	0.10
3	0.21	0.16	0.06	0.06	0.01	0.03
4	0.10	0.13	0.05	0.07	0.06	0.06
5						
Analyst 1	0.04	0.05	0.06	0.07	0.05	0.05
Analyst 2	0.08	0.09	0.07	0.07	0.09	0.09
6	0.10	0.12	0.14	0.18	0.16	0.10
7	0.06		0.05			
Sample 2						
1	0.19	0.18	0.16	0.14	0.11	0.12
2	0.27	0.28	0.21	0.19	0.25	0.27
3	0.40	0.48	0.17	0.16	0.13	0.12
4	0.32	0.32	0.18	0.22	0.12	0.12
5						
Analyst 1	0.20	0.20	0.19	0.20	0.18	0.18
Analyst 2	0.22	0.22	0.21	0.22	0.19	0.19
6	0.18	0.28	0.28	0.20	0.17	0.17
7	0.11		0.20			
Sample 3						
1	0.10	0.14	0.05	0.06	0.06	0.05
2	0.14	0.19	0.10	0.09	0.20	0.20
3	0.24	0.17	0.05	0.06	0.06	0.05
4	0.11	0.13	0.05	0.09	0.04	0.04
5						
Analyst 1	0.07	0.08	0.08	0.08	0.07	0.08
Analyst 2	0.09	0.09	0.08	0.08	0.08	0.08
6	0.09	0.09	0.14	0.14	0.05	0.05
7	0.04		0.02			
Sample 4						
1	0.11	0.09	0.07	0.05	0.04	0.04
2	0.15	0.17	0.12	0.11	0.16	0.14
3	0.12	0.16	0.16	0.14	0.05	0.05
4	0.13	0.15	0.12	0.12	0.05	0.05
5						
Analyst 1	0.07	0.06	0.09	0.09	0.07	0.07
Analyst 2	0.10	0.11	0.08	0.08	0.09	0.09
6	0.09	0.05	0.13	0.14	0.10	0.10
7	0.05		0.08			

RECOMMENDATIONS[1]

It is recommended—

(1) That the "Cold Test" remain as a tentative method and further work on the method be discontinued.

(2) That the combined Reichert-Meissl and Polenske method be made official and substituted for the present separate methods under "soluble" and "insoluble volatile" acids in *Methods of Analysis*, with the exception that the illustration of apparatus, page 292, be retained (second action).

(3) That the Kirschner method, using standard solutions of sodium, potassium or barium hydroxide for the titration, as described in the previous report, be made official (second action).

(4) That methods for the determination of moisture and volatile matter with particular reference to the hot plate method be further studied.

(5) That methods for the determination of ether insoluble hexabromide number of drying oils be studied.

REPORT ON BAKING POWDER AND BAKING CHEMICALS

By MAYNE R. COE (Food Research Division, Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

The report this year on baking powder describes work done by collaborators on a new method for determining available carbon dioxide and includes recommendations for changes in methods that are to be incorporated in the revision of *Methods of Analysis*.

The new method, which is submitted as a contributed paper,[2] affords a means of estimating the available carbon dioxide directly in much less time than is possible by the official gasometric method. The same apparatus is used but a 5 per cent ammonium sulfate solution is substituted for sulfuric acid (1+5); 25 cc. of this ammonium sulfate reagent is run into the decomposition flask containing 1.7 grams of baking powder, and the contents are heated to boiling for a few seconds and then cooled. The flask is heated again and cooled to room temperature by immersion in successive beakers of water. When there is no further change in volume of gas and the temperature of the water in the beaker is the same as that of the surrounding air, equilibrium is established. The volume of carbon dioxide is then noted.

The following table shows a comparison of results obtained by the proposed method and those obtained by the official gasometric method.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 58 (1931).
[2] *This Journal*, 14, 99 (1931).

COLLABORATOR	CARBON DIOXIDE	
	OFFICIAL METHOD	DIRECT METHOD
	per cent	*per cent*
Percy O'Meara, State of Michigan, Dept. of Agriculture	13.8	13.9
G. C. Richards, Jaques Mfg. Company	14.3	13.4
V. E. Mumsey, U. S. Food and Drug Adm.	14.3	14.3
J. R. Davies Calumet Baking Powder Company	13.1	12.7
C. M. Moore, Victor Chemical Works	13.6	12.6
J. Schlaeger, Victor Chemical Works	13.7	12.3
Herbert Oliver, Provident Chemical Works	13.9	12.7
Emanuel Kaplan, Johns Hopkins University	13.9	13.5
M. R. Coe	13.7	13.8

All collaborators who volunteered to comment on the merits of the proposed method gave favorable criticism. A number made helpful suggestions, which have been included in the contributed paper, and also expressed a willingness to do further collaborative work. As the table shows, six of the nine collaborators obtained appreciably lower results using the proposed method than they did when using the official method. This difference will be explained in a more detailed account of the procedure which will be included in the instructions to be sent out next year.

Because an error in the gasometric method was reported by the referee last year, J. R. Chittick did experimental work on the determination of total carbon dioxide in baking powder and read a paper covering his results at the meeting of the American Association of Cereal Chemists last May.[1] The purpose of this paper was to prove that there is no error in the published gasometric method as had been alleged by R. Hertwig and J. S. Hicks.[2]

RECOMMENDATIONS[3]

It is recommended—

(1) That further study be made of the tentative method for the determination of aluminum by precipitation with phenylhydrazine.

With regard to the revised *Methods of Analysis* it is recommended that the following changes be adopted:

(2) That in the Knorr method for the determination of total carbon dioxide, one additional sulfuric acid bulb be placed next to the Liebig condenser, and also that two additional potash bulbs be placed adjacent to those already present.

(3) That under the gasometric method for the determination of total carbon dioxide, the words "must be" be substituted for "consists of" in the passage, "The decomposition flask consists of a 250 cc." etc.; that af-

[1] *Cereal Chem.*, **7**, 473 (1930).
[2] *Ibid.*, **2**, 482 (1928).
[3] For report of Subcommittee C and action of the association, see *This Journal*, **14**, 59 (1931).

ter the words, "factor weight," "1.7 grams" be inserted; and that sulfuric acid (1+5) be used instead of hydrochloric acid (5+4).

(4) That the qualitative test for the determination of aluminum in the presence of phosphates be deleted and the following method[1] be substituted because this test is more sensitive and less time consuming.

REAGENTS

(a) *Hydrochloric acid normal solution.*—Dilute 100 cc. of strong HCl to 1 liter with water.

(b) *Ammonium acetate 3 N solution.*—Dissolve 23.1 grams in water and dilute to 100 cc.

(c) *Aurintricarboxylic acid.*—Dissolve 0.1 gram of the acid in water and dilute to 100 cc.

DETERMINATION

Dissolve 1–5 grams of baking powder in 5 cc. of normal hydrochloric acid and 5 cc. of 3 *N* ammonium acetate; add 5 cc. of 0.1 per cent solution of aurintricarboxylic; mix; allow the lake formation to take place; and make the solution alkaline with concentrated ammonium hydroxide containing a small quantity (approximately 0.1–1.0 gram) of ammonium carbonate. A bright persistent red precipitate indicates the presence of aluminum.

(5) That the method for the determination of fluorides and also the apparatus given on p. 312, pars. **35** to **38,** be deleted and the proposed method and apparatus described by W. H. Ross be substituted as tentative, because the old method and apparatus are obsolete. The author and also his co-workers have made hundreds of analyses, and they have found the method very satisfactory. It has been published.[2]

[1] *J. Am. Chem. Soc.*, **47**, 142 (1928).
[2] *This Journal*, **11**, 231 (1928).

CONTRIBUTED PAPERS

A STUDY OF TRAVERS' METHOD FOR THE ESTIMATION OF FLUORINE WITH REFERENCE TO INSECTICIDES

By C. M. SMITH, E. H. HAMILTON, and J. J. T. GRAHAM (Insecticide Control, Food and Drug Administration,[1] Washington, D. C.)

Sodium fluoride and sodium silicofluoride have long been used as ingredients of household insecticides designed for the control of ants and roaches, and in the past few years the silicofluoride and other fluorine compounds have been used in agricultural insecticides. The determination of the total fluorine content of such preparations has therefore become of considerable importance to the insecticide chemist.

The accurate estimation of fluorine has been one of the difficult problems of inorganic chemistry, and hence has been the object of many investigations. A comprehensive review of methods has been given by Wagner and Ross[2] and more recently by Gmelin-Kraut.[3] Among these are the evolution methods, in which the fluorine is first volatilized as silicon tetrafluoride, which is then recovered and estimated in one of various ways, e.g. in Carnot's method[4] it is caught in a solution of potassium fluoride and the potassium silicofluoride formed is recovered and weighed.

It was not until 1921, however, that Travers[5] showed that silica could be converted directly into silicofluoride by treatment with a soluble fluoride and acid in aqueous solution. The reactions:

$$6\,NaF + SiO_2 + 4HCl = Na_2SiF_6 + 4NaCl + 2H_2O$$
$$Na_2SiF_6 + 2KCl = K_2SiF_6 + 2NaCl$$
$$K_2SiF_6 + 4KOH = 6KF + SiO_2 + 2H_2O$$

were first employed by him to determine silica, and were later adapted[6] to the estimation of fluorine. He tested the method against pure potassium acid fluoride, KHF_2, and obtained excellent results. Since a search of the literature has not revealed any study of this method except that by Travers himself, the following work is presented in the hope that it will prove of value to other analysts.

Travers' recommendations may be summarized briefly as follows: The alkaline fluoride is mixed at room temperature with a solution of potassium silicate containing about twice as much silica as is necessary according to the first equation given. The mixture is then just acidified (methyl orange) with strong hydrochloric acid, 2 cc. excess of this reagent is added, and then enough potassium chloride is dissolved in the mixture to form

[1] This work was begun by Smith and Hamilton while they were employed by the Food and Drug Administration. Following their transfer to the Bureau of Chemistry and Soils and the Bureau of Standards, respectively, the investigation was extended by Graham.

[2] *Ind. Eng. Chem.*, 9, 1116 (1917).

[3] Handbuch der anorganischen Chemie, Fluor (1926).

[4] *Bull. Soc. Chim.*, (3) 9, 71 (1893).

[5] *Compt. rend.*, 173, 714 (1921).

[6] *Ibid.*, 836.

a 20 per cent solution. The potassium silicofluoride that forms is then filtered, washed, and titrated with standard alkali.

While the precipitation of silica from potassium silicate in the reaction mixture probably is the best way to facilitate the formation of silicofluoride, the writers found its use objectionable. The unused silica is so gelatinous that difficulty is experienced in filtering and washing the precipitate preparatory to titration. When it was found that powdered quartz was not sufficiently reactive, precipitated silica was tried, and it proved to be very satisfactory in that it filtered well and still possessed a rate of reaction sufficient for the purpose of the method. Later, powdered silica gel was found to be just as suitable and was adopted for the remainder of the experiments.

The method first used by the writers differed from Travers' method in the kind of silica used, in the use of a lesser excess (only about 0.5 cc.) of strong hydrochloric acid and in the addition of alcohol to the media and wash solution. It was first checked against two samples of sodium fluoride, one of which (A) was purchased as C. P. and the other (B) made by five recrystallizations from a commercial sodium fluoride of ordinary commercial grade. Both preparations were subjected to very careful qualitative tests for all the probable impurities and found to be free from them. In addition one of them was analyzed by the calcium fluoride precipitation method. The results obtained are shown in Table 1.

TABLE 1.

Fluorine in sodium fluoride.

SAMPLE	THEORETICAL	BY CaF_2 METHOD	TRAVERS' METHOD
	per cent	*per cent*	*per cent*
A	45.24	44.91	44.91
		45.06	45.01
B	45.24		44.91
			44.82
			45.11
			44.97

The results by Travers' method are all slightly below the theoretical value, but they check the CaF_2 method, and it was therefore felt that they were entirely satisfactory. Since a larger supply of Sample A was available, it was used for the following experiments. Its purity was considered, for purposes of comparison, to be 99.4 per cent, corresponding to 44.97 per cent fluorine.

Some chicken-lice and roach powders consisting only of commercial sodium fluoride containing as impurities sodium carbonate, sodium sulfate, sodium bifluoride and sodium silicofluoride, are encountered on the mar-

ket. These normal constituents evidently cause no interference. The majority of products, however, are mixtures of fluorine compounds with other insecticidal materials or with diluents. Attention was therefore directed to an investigation of the effect on the method of certain of such compounds.

In attempting to determine the fluorine in known mixtures of sodium fluoride and calcium carbonate it was found that the latter reacted rather slowly with the hydrochloric acid in the cold, and consequently it was difficult to reach and maintain the slight acidity necessary to the first reaction of the process. Low results were obtained due to the alkaline reaction of unchanged calcium carbonate in the titration stage. This difficulty was overcome by heating the mixture just to boiling with an excess of strong acid (about 0.5 cc.) during the formation of the silicofluoride. A test of this modification on pure sodium fluoride gave results equally as good as those obtained without the modification, thus disposing of the fear that some silicon tetrafluoride might be lost during the heating. Experiments were then made on known mixtures of the pure sodium fluoride with various other compounds. No stock mixtures were made, the two components being weighed out separately for each determination. Table 2 contains the data concerning these experiments.

TABLE 2.

Fluorine in the presence of various other materials.

WT. OF NaF TAKEN	WT. OF OTHER MATERIAL	WT. OF F TAKEN	WT OF F FOUND	RECOVERY
gram	*gram*	*gram*	*gram*	*per cent*
0.8	0.1 $CaCO_3$	0.3598	0.3578	99 4
0.8	0.1 "	0.3598	0 3595	99.9
0.8	0.1 "	0.3598	0.3574	99 3
0.8	0.1 $Ba(OH)_2$ $8H_2O$	0.3598	0.3574	99.3
0.8	0.1 "	0.3598	0.3583	99 6
0.8	0.1 $Na_2HAsO_4 \cdot 7H_2O$	0.3598	0.3578	99.4
0.8	0.1 "	0.3598	0.3587	99.7
0.8	0.1 Com'l. Calcium Ars.	0.3598	0.3557	98.9
0.8	0.1 " " "	0.3598	0.3570	99.2
0.8	0.2 $AlCl_3 \cdot 6H_2O$	0.3598	0.2807	78.0
0.8	0.2 "	0.3598	0.2803	77.9
0.7	0.2 "	0.3148	0.2438	77.4
0.7	0.2 $KAl(SO_4)_2 \cdot 12H_2O$	0.3148	0.2857	90 8
0.8	0.2 $FeCl_3 \cdot 6H_2O$	0.3598	0.3498	97.2
0.8	0.2 "	0.3598	0.3553	98.7
0.8	0.2 "	0.3598	0.3536	98.3
0.7	0.2 "	0.3148	0.2963	94.1

An examination of Table 2 will show that while the results are quite low in the presence of aluminum chloride, and, to a less extent, of ferric chloride, they are in the other cases very good, and probably as accurate as could be obtained by any of the more laborious methods involving preliminary separation of the fluoride from the interfering substance. The interference by iron and aluminum is probably due in some way to the formation of the insoluble complex fluorides, Na_3FeF_6 and Na_3AlF_6.[1] It is noted that the fluorine lost in two of the cases in which aluminum chloride was used corresponds almost exactly to the quantity necessary to form Na_3AlF_6.

Borax is very frequently mixed with sodium fluoride to make proprietary insecticides. The results obtained on mixtures of these two salts, given in Table 3, deserve special attention.

TABLE 3

Fluorine in the presence of borax.

NaF	Borax	F	F found	Recovery
gram	*gram*	*gram*	*gram*	*per cent*
0.8	0.2	0.3598	0.2863	79.6
0.8	0.2	0.3598	0.2863	79.6
0.8	0.95	0.3598	0.1274	35.4
0.8	0.95	0.3598	0.1274	35.4

The results in Table 3 show that the method is worthless in the presence of borax. That the low results were caused by the formation of potassium borofluoride, KBF_4, was shown as follows: An experiment similar to the above was carried through except that no silica was used. A crystalline insoluble material that contained both boron and fluorine was obtained on filtering. When titrated it reacted only very slowly with the alkali, and the titration recorded was equivalent to only 2.4 per cent of the fluorine originally used. This action agrees with the known behavior of potassium borofluoride. The authors are at a loss to explain Travers' statement[2] that "the method . . . has already given excellent results for the determination of fluorine in . . . KBF_4 . . . ," especially since in a later article he and Malaprade[3] report as follows: "The preceding facts explain the necessity of a preliminary alkaline fusion for the determination of fluorine in fluoborates of the type MBF_4, even in the case of a fluoborate soluble in water, as in the case of sodium, by the fluosilicate method described by one of us."

A few attempts were made to devise a method of overcoming the deleterious action of borax. The following procedures were tried.

[1] Travers, *Compt. rend.*, 185, 893, 1043 (1927).
[2] *Ibid.*, 173, 836 (1921).
[3] *Compt. rend.*, 187, 891 (1928).

1. The mixture of borax and sodium fluoride was covered with methyl alcohol, a small quantity of strong hydrochloric acid was added, and the whole was evaporated to dryness in an attempt to expel the boron as methyl borate. The evaporation was repeated once or twice and the residue treated by the modified Travers' method.

2. Same as 1 except that the evaporations were not carried to dryness, but only to a volume of 2 or 3 cc.

3. The sample was covered with 20 cc. of methyl alcohol, 1 cc. of glacial acetic was added, the mixture was allowed to stand 1 hour with frequent stirring, then filtered and washed with methyl alcohol, after which the residue was subjected to the modified Travers' method. It was hoped that the boric acid liberated from the borax by the acetic acid would dissolve in the alcohol and be filtered off from the sodium fluoride.

The results obtained are given in Table 4.

TABLE 4.

Results of special treatment of samples containing borax.

SODIUM FLUORIDE	BORAX	PROCEDURE		F TAKEN	F FOUND	RECOVERY OF F
gram	*gram*	*No.*	*Evaporations*	*gram*	*gram*	*per cent*
0.8000	0.2	1	2	0.3598	0.3391	94.2
0.8000	0.2	1	3	0.3598	0.3400	94.5
0.8000	0.8	2	3	0.3598	0.3472	96.5
0.8000	0.8	2	3	0.3598	0.3480	96.7
0.8000	0.8	2	4	0 3598	0.3229	89.7
0.8000	0.8	3		0.3598	0.3474	96.6
0.8000	0.8	3		0.3598	0.3382	94.0

It is seen that all these preliminary treatments have greatly improved the results, but they are still unsatisfactory.

The method outlined was tested in the presence of lime (calcium oxide, U. S. P., being used) and it was found that while 0.1 gram caused only very slight errors, the presence of 0.2 and 0.3 gram caused large departures from the correct value. These errors were probably caused by the formation of calcium fluoride, which was then not completely converted to silicofluoride. In order to overcome this difficulty the method was again modified slightly by increasing the excess of strong acid to 2 cc. and boiling the acidified solution for 1 minute in a covered beaker. An improved method of filtration was also adopted. The method as finally modified is as follows:

TOTAL FLUORINE

REAGENTS

(a) *Alcoholic Potassium Chloride Solution.*—Dissolve 60 grams of KCl in 400 cc. of distilled water, add 400 cc. of 95 per cent ethyl alcohol, and test with phenolphthalein; if the solution is not neutral, adjust it to exact neutrality by the addition of sodium hydroxide or hydrochloric acid.

(b) *Standard Sodium Hydroxide.*—Prepare an approximately 0.2 *N* solution of NaOH in a manner to assure the absence of carbonate.

DETERMINATION

Treat 0.5000 gram of sample in a small beaker with 20–25 cc. of distilled water. Add 0.3 gram of finely divided *precipitated* silica and a few drops of methyl orange indicator. Add strong hydrochloric acid drop by drop until the solution assumes an apparently permanent pink color, after which add 2 cc. in excess, cover the beaker with a watch-glass, and boil for 1 minute. Cool to room temperature, add 4 grams of solid potassium chloride, and stir until the latter dissolves. Next add 25 cc. of 95 per cent ethyl alcohol and let stand for 1 hour with frequent stirring. Filter through a Gooch crucible[1] containing a disc of filter paper covered by a medium pad of asbestos. Wash the precipitate with the alcoholic potassium chloride solution (a) until one washing does not destroy the color made by 1 drop of 0.2 *N* sodium hydroxide and phenolphthalein. (Three or four washings are usually sufficient.) Transfer the crucible and contents to a 400 cc. beaker, add 100 cc. of recently boiled water and 1–2 cc. of 1 per cent phenolphthalein solution, heat, and titrate with the standard sodium hydroxide solution (b). Finish the titration with the fluoride solution actively boiling. Calculate the percentage of fluorine present on the basis that 1 cc. of 0.2 *N* sodium hydroxide solution is equivalent to 0.005700 gram of fluorine.

In the following work it was necessary to use a new sample of sodium fluoride. Its purity was checked by the first modification of Travers' method used by the writers and by the Bureau of Standards' modification of Starck's lead chlorofluoride method.[2] The results are shown in Table 5.

TABLE 5

Comparative analyses of sodium fluoride

(Results expressed as percentage of fluorine)

Lead chlorofluoride method	44.98		45.08
Travers' method as first modified	45.04		45.15
Travers' method as finally modified	44.98		45.10
Average		45.06	

TABLE 6.

Fluorine in the presence of lime.

(0.5 gram of sodium fluoride, containing 0.2253 gram of fluorine)

CaO	ORIGINAL MODIFICATION		FINAL MODIFICATION	
	FLUORINE FOUND	RECOVERY	FLUORINE FOUND	RECOVERY
gram	*gram*	*per cent*	*gram*	*per cent*
0.1	0.2238	99.3	0.2249	99.8
0.1	0.2238	99.3	0.2249	99.8
0.2	0.1903	84.5	0.2249	99.8
0.2	0.1883	83.6	0.2255	100.1
0.3	0.1271	56.4	0.2249	99.8
0.3	0.1431	63.5	0.2249	99.8

[1] Fritted glass crucibles were tried for this filtration, but they were unsatisfactory because some of the precipitate was held in the pores of the fritted glass so tenaciously that even boiling for several minutes failed to give a complete reaction between the silicofluoride and the standard alkali.

[2] Lundell and Hoffman, Bur. Standards Research Paper 110 (1929).

The improvement in the results obtained by using this last modification is well shown in Table 6, in which the results obtained by it in the presence of lime are compared with those obtained by the former modification.

The results obtained in the presence of numerous other materials commonly found mixed with the fluorine compounds in proprietary insecticides are given in Table 7. No determinations were made with borax as it was felt that the changes introduced in the method would produce no better results.

TABLE 7.

Fluorine in the presence of various other materials.

(Theory for F, 0.2253 gram)

MATERIAL ADDED		F FOUND	RECOVERY	MATERIAL ADDED		F FOUND	RECOVERY
	gram	*gram*	*per cent*		*gram*	*gram*	*per cent*
Sulfur	0.1	0.2244	99.6	Paradichlorobenzene	0.1	0.2233	99.1
	0.3	0.2244	99.6		0.3	0.2239	99.4
	0.5	0.2244	99.6		0.5	0.2236	99.2
Starch	0.1	0.2236	99.2	Naphthalene	0.1	0.2233	99.1
	0.3	0.2233	99.1		0.3	0.2239	99.4
	0.5	0.2236	99.2		0.5	0.2239	99.4
Flour	0.1	0.2236	99.2	Potassium alum	0.1	0.2184	96.9
	0.3	0.2233	99.1	$KAl(SO_4)_2 \cdot 12H_2O$	0.3	0.2083	92.5
	0.5	0.2236	99.2		0.5	0.1968	87.4
Pyrethrum	0.1	0.2244	99.6	Aluminum chloride	0.1	0.2144	95.2
	0.3	0.2268	100.7	$AlCl_3$ $6H_2O$	0.3	0.1927	85.5
	0.5	0.2291	101.7		0.5	0.1725	76.6
Talc	0.1	0.2239	99.4	Ferric chloride	0.1	0.2239	99.4
	0.3	0.2239	99.4	$FeCl_3 \cdot 6H_2O$	0.3	0.2225	98.8
	0.5	0.2239	99.4		0.5	0.2227	98.8
Kaolin	0.1	0.2163	96.0	Cresols	0.1 cc.	0.2233	99.1
	0.3	0.2111	93.7		0.3 cc.	0.2233	99.1
	0.5	0.2088	92.7		0.5 cc.	0.2233	99.1
Diatomaceous earth (Celite #2)	0.1	0.2242	99.5	Coal tar neutral oils	0.1 cc.	0.2230	99.0
	0.3	0.2204	97.8		0.3 cc.	0.2236	99.2
	0.5	0.2187	97.1		0.5 cc.	0.2239	99.4
Tobacco	0.1	0.2244	99.6	Calcium arsenate	0.1	0.2253	100.0
	0.3	0.2251	99.9	Commercial	0.3	0.2250	99.9
	0.5	0.2253	100.0		0.5	0.2250	99.9
Paris green	0.1	0.2239	99.4	Acid lead arsenate	0.1	0.2274	100.9
	0.3	0.2244	99.6		0.3	0.2311	102.6
	0.5	0.2239	99.4		0.5	0.2305	102.3

The ingredients listed in Table 7 fall into three groups: those that cause no appreciable interference; those that cause high results; and those that cause low results. The first group consists of sulfur, starch, flour, tobacco, talc, paradichlorobenzene, naphthalene, cresols, coal tar neutral oils, Paris green, and calcium arsenate. Pyrethrum and lead arsenate comprise the second group, which has a tendency toward high results. The error in the case of pyrethrum results perhaps from the formation of insoluble organic acids by hydrolysis during the boiling. The error caused by lead arsenate is due to insoluble lead chloride which reacts with the standard alkali to form lead hydroxide. The third group includes ferric chloride, aluminum chloride, potassium alum, kaolin, and diatomaceous earth. The results with the ferric salt are better than those obtained before and are only slightly in error, but the results with the aluminum salts are still hopelessly low. The errors caused by kaolin and diatomaceous earth are undoubtedly due to a partial solution of their aluminum content.

SUMMARY AND CONCLUSIONS

A modification of Travers' method for the determination of fluorine was developed and found satisfactory for the analysis of sodium fluoride alone and in mixture with many of the ingredients ordinarily found in proprietary insecticides.

Iron causes slightly low results, and appreciable quantities of aluminum and boron compounds render the method useless. Pyrethrum powder causes slightly high and lead arsenate somewhat higher results.

The method given is more rapid than most of the other methods in common use and equally as accurate.

METHOD FOR THE DETERMINATION OF LEAD AND COPPER IN BORDEAUX-LEAD ARSENATE MIXTURES[1]

By JOHN C. BUBB (U. S. Food and Drug Administration, New York City)

Bordeaux mixture with lead arsenate is a combined insecticide and fungicide of great importance. The determinations usually made in the analysis of this product are moisture, carbon dioxide, total arsenic, water-soluble arsenic, lead oxide and copper. The Association of Official Agricultural Chemists has adopted official methods for all these determinations, and when carefully followed they give accurate results. The method for lead oxide and copper, however, requires considerable time and manipulation, and therefore the author was led to investigate other procedures. The fact that lead arsenate is insoluble and copper and calcium compounds are soluble in dilute acetic acid suggested the use of this acid as a means of separating the lead and copper in such a mixture.

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1930.

In the earlier experiments some good results were obtained by the simple addition of dilute acetic acid to make the separation, but occasionally samples were encountered in which the separation was not complete. Small crystals of blue vitriol, and other copper compounds that were apparently occluded with the lead arsenate, resisted the action of this weak acid. Heating the residue after filtration with a small quantity of nitric acid and more dilute acetic acid was found to correct this deficiency, and this modification suggested the present method, which was found to give accurate results on all samples.

The following procedure was finally adopted:

Treat 1.0 gram of the sample in a 250 cc. beaker with 50 cc. of acetic acid solution (1+2) and heat the mixture on the steam bath for 5–10 minutes. Add 0.5 gram of calcium arsenate to insure the presence of an excess of arsenic oxide, which is required to convert any tri-lead arsenate [$Pb_3(AsO_4)_2$] or other lead compound into the acid arsenate ($PbHAsO_4$), and then add concentrated nitric acid drop by drop with stirring until any blue coloration in the insoluble residue clears up and the lead arsenate is white. (2 or 3 drops to 1.5 cc. of HNO_3 will be required. Note amount of acid used, and do not add any further excess.) Stir, and continue heating for a few minutes. Cool the mixture in a water bath and nearly neutralize the nitric acid present with strong ammonium hydroxide, adding an amount exactly equal to the amount of HNO_3 that was used. (Only a slight excess of mineral acid will be left.) Stir, and allow to stand at room temperature for 20 minutes.

Filter the mixture through a No. 44 Whatman filter paper, collecting the filtrate, which contains the copper and calcium salts, in a 500 cc. Erlenmeyer flask. Wash the insoluble acid lead arsenate with small portions of hot water until no copper is left on the filter.

Lead: Transfer the filter paper to the original beaker and dissolve the lead arsenate in 25 cc. of nitric acid (1+4) by heating. Filter into a 600 cc. beaker, wash with hot water, and make the volume to at least 400 cc. Then proceed with the determination of lead as chromate according to the official method.[1] Calculate to PbO.

Copper: Add to the original cooled filtrate containing copper, a few more drops of strong ammonium hydroxide to neutralize completely the nitric acid that was added. Then add 3 grams of potassium iodide and titrate the liberated iodine with standard sodium thiosulfate solution (1 cc. = 0.005 gram of Cu).

A few of the comparative results obtained are given in the table.

As previously mentioned some samples of Bordeaux-lead arsenate contain acetic acid-insoluble forms of copper, and it is necessary to add nitric acid to change these to the soluble forms; otherwise there will be a loss of copper. The nitric acid added must be kept down to the minimum quantity that will completely clear the insoluble residue from particles of copper compounds and leave an acid condition favorable to the precipitation of acid lead arsenate. The lead arsenate must be white and granular and contain no blue coloration when filtered and washed.

Some Bordeaux lead arsenates also contain calcium arsenate. In such cases the addition of calcium arsenate specified in the method may be omitted.

[1] *Methods of Analysis, A.O.A.C.*, 1925, 58.

Analyst	Lead Oxide		Copper (Cu)	
	Official Method per cent	Proposed Method per cent	Official Method per cent	Proposed Method per cent
C. F. Cressy	10.39	10.17	11.18	11.16
	10.40	10.34		
	Av. 10.40	Av. 10.26		
E. C. Haas	16.35	16.60	18.60	18.48
	16.77	16.55		
	Av. 16.56	Av. 16.58		
Leslie Hart	A— 31.75	31.80		
		31.81		
	B— 28.99	29.06		
		29.14		
		Av. 29.10		
W. L. Miller	12.42	12.24	11.19	11.31
	12.25	12.29	11.26	11.28
	Av. 12.34	Av. 12.27	Av. 11.23	Av. 11.30
J. C. Bubb	12.31	12.18		
	12.31	12.21		
		Av. 12.20		
	10.13	10.13		
	10.21	10.13		
	Av. 10.17			
	15.63	15.64	12.43	12.44
	15.59	15.52	12.49	12.53
	Av. 15.61	Av. 15.58	Av. 12.46	Av. 12.49
	18.01	18.00	8.76	8.68
	17.93	17.89		8.77
	Av. 17.97	Av. 17.95		Av. 8.73

The method is rapid, requiring much less time to carry out than the official method. The results are quite accurate, and they compare very favorably with those obtained by any other method with which the author has had experience.

THE SOLUBILITY OF PHOSPHATES IN NEUTRAL AMMONIUM CITRATE SOLUTION[1]

By K. D. JACOB, K. C. BEESON, L. F. RADER, JR., and W. H. ROSS
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Recent investigations indicate the desirability of a change in the official method (16)[2] for the determination of available phosphoric acid in water-insoluble phosphates, particularly those formed by the treatment of superphosphate with ammonia. Keenen (15), Howes and Jacobs (9), and Jacob, Hill, Ross and Rader (13) have shown that treatment of superphosphate with ammonia results in the conversion of a portion of the water-soluble phosphoric acid into water-insoluble compounds, the amount of the latter increasing with the quantity of ammonia added. These investigators have also shown—(1) that the water-insoluble phosphates formed under these conditions consist principally of di- and tricalcium phosphates, the amount of the latter increasing with the quantity of ammonia added; (2) that the solubility in neutral ammonium citrate solution of the water-insoluble phosphoric acid present in ammoniated superphosphates containing approximately 3–6 per cent of ammonia varies with the weight of sample taken for analysis, the variation being greater in the more highly ammoniated materials; and (3) that these variations are due principally to the presence of the rather difficultly soluble tricalcium phosphate.

Recent vegetation tests (19) with tricalcium phosphate and with citrate-insoluble residues extracted from highly ammoniated superphosphates according to the present official method indicate that these materials have a much greater effect in promoting the growth of plants than would be expected on the basis of their citrate solubility as determined by treating 2 grams of the materials with 100 cc. of neutral ammonium citrate solution. The agreement between the fertilizer value of these materials, as shown by vegetation tests, and their solubility in citrate solution becomes closer as the weight of sample is decreased, resulting in correspondingly lower figures for citrate-insoluble phosphoric acid.

It seems therefore that a change in the official method for the determination of available phosphoric acid should be made, and that in so far as it concerns tricalcium phosphate and ammoniated super phosphates a satisfactory correlation between the actual fertilizer value of these materials and their solubility in citrate solution can be obtained simply by decreasing the weight of sample taken for analysis. In this connection it then becomes important to know how the citrate solubility of other water-

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists, October, 1930.
[2] Numerals in parentheses refer to "Literature Cited" at the end of the paper.

insoluble phosphates is affected by varying the weight of sample. In order to obtain information on this subject, the relation between the weight of sample and the quantity of phosphoric acid dissolved by 100 cc. of neutral ammonium citrate solution was determined on 64 samples of various types of phosphatic materials, 0.5, 1.0, 1.5 and 2.0 gram portions being taken for analysis. The materials investigated included di- and tricalcium phosphates, magnesium, iron and aluminum phosphates, various bone products, acidulated and non-acidulated natural phosphates, basic slags and calcined phosphates.

METHOD OF DETERMINING CITRATE-INSOLUBLE PHOSPHORIC ACID

When tricalcium phosphate, highly ammoniated superphosphates and finely divided natural phosphates are treated with citrate solution according to the official method, clear filtrates are difficult to obtain because a portion of the phosphate usually passes through the filter paper in the colloidal condition, thus resulting in low values for citrate-insoluble phosphoric acid. Clear filtrates from such materials were obtained, however, by the use of short Pasteur-Chamberland filter tubes (grade F). Duplicate results thus obtained were usually in excellent agreement and, in the case of phosphates which filtered clear through paper, the method gave results that checked closely with those obtained by the use of filter paper. These tubes were used in obtaining all the figures for citrate-insoluble phosphoric acid given in the present paper. The details of their use for this purpose will be discussed in a later paper.

Except for the method of filtration and variations in the weight of sample the official procedure was closely followed. Except as noted otherwise, all the samples were ground to pass a 100-mesh sieve. Water-soluble phosphoric acid, when present, was removed by washing with cold water just prior to the citrate digestion. The citrate solution was carefully prepared according to the official method (16), phenol red being used as an indicator. The pH values of the several solutions used ranged from 6.9 to 7.0, as determined potentiometrically by means of the hydrogen electrode.[1]

SYNTHETIC CALCIUM PHOSPHATES

The relation between the weight of sample and the quantity of phosphoric acid dissolved by 100 cc. of citrate solution was determined on 10 samples of synthetic di- and tricalcium phosphates, calcium hydroxyphosphate and calcium pyrophosphate. The content of total phosphoric acid and calcium oxide and the phosphoric acid-calcium oxide ratios in these samples are given in Table 1. Their source, method of preparation, etc., are described briefly as follows:

[1] The potentiometric pH measurements were kindly made by E. F. Snyder of the Soil Fertility Division

Dicalcium Phosphates.—Sample No. 387 was Baker and Adamson's C. P. material. It appeared to be in the amorphous condition, and the analysis indicates that it was the anhydrous salt. Sample No. 390 was Kahlbaum's C. P. crystalline material containing approximately 2 moles of water of crystallization. Both these samples contained slightly more calcium oxide than is theoretically required for the pure salt. Sample No. 1021 was a commercial fertilizer material manufactured by neutralizing a hydrochloric acid solution of Florida pebble phosphate with lime.

TABLE 1.
Composition of calcium phosphates.

Sample	P_2O_5	CaO	P_2O_5—CaO Ratio
	DICALCIUM PHOSPHATE*		
	per cent	*per cent*	
387	50.45	40.25	1.253
390	42.29	34.02	1.243
1021	40.20	36.73	1.094
	TRICALCIUM PHOSPHATE†		
287	40.86	49.50	0.825
1023	40.44	50.25	0.805
1093	41.82	51.22	0.816
1094	45.38	54.36	0.835
1095	40.48	47.46	0.853
	CALCIUM HYDROXYPHOSPHATE‡		
1117	38.68	49.99	0.774
	CALCIUM PYROPHOSPHATE*		
1025	55.56	44.55	1.247

* Theoretical P_2O_5-CaO ratio = 1.267.
† Theoretical P_2O_5-CaO ratio = 0 845.
‡ Theoretical P_2O_5-CaO ratio = 0 760.

Tricalcium Phosphate.—Samples Nos. 287, 1023, and 1093 were C. P. materials sold by Eimer and Amend, Baker and Adamson, and Merck, respectively. All these samples contained more calcium oxide than is theoretically required for the pure salt. Sample No. 1094 was prepared by neutralizing a water suspension of pure calcium hydroxide with a dilute solution of pure phosphoric acid, evaporating to dryness on the steam bath, and finally heating at 900–950°C., as described by Jacob and Reynolds (10). Sample No. 1095 was prepared by slowly adding a solution of pure trisodium phosphate to a solution containing an excess of calcium nitrate. The precipitate was washed with a saturated solution of trical-

TABLE 2.

Citrate-insoluble phosphoric acid in synthetic calcium phosphates.

SAMPLE	P_2O_5					PERCENTAGE OF TOTAL P_2O_5 PRESENT AS CITRATE-INSOLUBLE P_2O_5			
	TOTAL	CITRATE-INSOLUBLE							
		WT. OF SAMPLE				WT. OF SAMPLE			
		0.5 g.	1.0 g.	1.5 g.	2.0 g.	0.5 g.	1.0 g.	1.5 g.	2.0 g.
	per cent	*per cent*	*per cent*	*per cent*	*per cent*				
DICALCIUM PHOSPHATE									
387	50.45	0.00	0.00	2.48	8.12	0.0	0.0	4.9	16.1
390	42.29	0.00	0.00	0.36	2.33	0.0	0.0	0.9	5.5
1021	40.20	6.07	6.87	7.78	8.91	15.1	17.1	19.4	22.2
TRICALCIUM PHOSPHATE									
287	40.86	14.54	23.60	28.40	31.17	35.6	57.8	69.5	76.3
1023	40.44	7.67	20.46	26.82	28.61	19.0	50.6	66.3	70.7
1093	41.82	14.06	24.99	29.36	32.05	33.6	59.8	70.2	76.6
1094	45.38	10.24	24.43	30.96	34.65	22.6	53.8	68.2	76.4
1095	40.48	7.14	19.48	23.30	26.38	17.6	48.1	57.6	65.2
CALCIUM HYDROXYPHOSPHATE									
1117	38.68	26.85	31.69	33.80	34.35	69.4	81.9	87.4	88.8
CALCIUM PYROPHOSPHATE									
1025	55.56	51.97	—	—	53.89	93.5	—	—	97.0

cium phosphate until the filtrate gave no test for nitrates, the salt finally being dried at a temperature of 50°C.

Calcium Hydroxyphosphate.—This material was obtained as the residue from the hydrolysis of tricalcium phosphate by neutral ammonium citrate solution. Jacob, Hill, Ross and Rader (13) have shown that the composition of the residue obtained by treating tricalcium phosphate with this reagent approaches that of calcium hydroxyphosphate, $(3Ca_3(PO_4)_2 \cdot Ca(OH)_2)$, and they have described the preparation of the particular sample used in the present investigation.

Calcium Pyrophosphate.—This material was prepared by heating dicalcium phosphate, No. 390, to constant weight at 800°C.

The figures given in Table 2 show that pure dicalcium phosphate is completely soluble in citrate solution when the weight of sample does not exceed 1 gram, but with larger samples a portion of the phosphoric acid is insoluble under the conditions of the experiments, the quantity increasing with the weight of the sample. Although higher percentages of citrate-insoluble phosphoric acid were obtained from the 1.5 and 2.0 gram samples of the anhydrous dicalcium phosphate, No. 387, than from the hydrated material, No. 390, under corresponding conditions, it will be noted that the anhydrous material originally contained a much higher percentage of total phosphoric acid, and larger actual weights of phosphoric acid were dissolved from it. The behavior of the commercial dicalcium phosphate, No. 1021, towards ammonium citrate solution indicates that this material contained some undecomposed phosphate rock. The results obtained on these samples confirm those obtained by Haskins (5), (6), (7) in his studies on the citrate solubility of "precipitated" phosphate.

In the case of tricalcium phosphate, there is a progressive and significant decrease in the percentage of citrate-insoluble phosphoric acid when the weight of sample is decreased by 0.5 gram steps from 2.0 to 0.5 gram, the change being greater when the weight of sample is decreased from 1.0 to 0.5 gram than when it is decreased from 2.0 to 1.0 gram. The results indicate that approximately 65–75 per cent of the total phosphoric acid in tricalcium phosphate is insoluble in citrate solution, under the conditions prescribed by the official method, when 2.0 gram samples are used, but when the weight of sample is reduced to 0.5 gram, only about 18–36 per cent of the total phosphoric acid is insoluble.

On the basis of the actual weights of phosphoric acid dissolved, calcium hydroxyphosphate is only about one-third as soluble as tricalcium phosphate, and the percentage of citrate-insoluble phosphoric acid is not greatly affected by decreasing the weight of sample from 2.0 to 0.5 gram.

Calcium pyrophosphate prepared by calcining dicalcium phosphate is quite insoluble in citrate solution, and a significant change in the percent-

age of citrate-insoluble phosphoric acid does not occur when the weight of sample is decreased. Calcium pyrophosphate prepared from pyrophosphoric acid or by precipitation from solutions of soluble pyrophosphates would very likely show a considerably higher citrate solubility.

Comparison of the quantities of phosphoric acid dissolved by 100 cc. of citrate solution indicates that as the weight of sample is increased beyond 0.5 gram the weight of phosphoric acid dissolved from tricalcium phosphate, calcium hydroxyphosphate and calcium pyrophosphate rapidly approaches the maximum quantity that can be dissolved from these phosphates under the conditions of the determinations. This is not true in the case of dicalcium phosphate where the weight of phosphoric acid dissolved is roughly proportional to the weight of sample, up to 2.0 gram.

BONE PRODUCTS

The bone products used in this investigation were commercial materials, except sample No. 1099, which was prepared in the laboratory by steaming sample No. 1123 under 40 pounds pressure for 30 days.

The results given in Table 3 show that the behavior of raw bone meal, steamed bone meal and naphtha-extracted bone when digested with citrate solution is similar to that of tricalcium phosphate (Table 2). Prolonged steaming under pressure decreases the solubility of bone in neutral ammonium citrate solution. Bone ash is quite insoluble in citrate solution, and the percentage of citrate-insoluble phosphoric acid in this material does not undergo a significant change when the weight of sample is decreased from 2.0 to 0.5 gram. Comparison of the quantities of phosphoric acid dissolved by 100 cc. of citrate solution indicates that as the weight of sample is increased beyond 0.5 gram the weight of phosphoric acid dissolved from bone rapidly approaches the maximum quantity that can be dissolved from this material under the conditions of the determinations.

Although the behavior of bone towards neutral ammonium citrate solution is quite similar to that of tricalcium phosphate, Shear and Kramer (20) state that there is no evidence to show the existence of tricalcium phosphate, $Ca_3(PO_4)_2$, in bone. As a result of x-ray investigations De Jong (2) concludes that bone is essentially a carbonato-phosphate, probably having the formula $3Ca_3(PO_4)_2 \cdot CaCO_3$. Gassmann (3) also concludes that bone is essentially a carbonato-phosphate containing 6 mols of water, $3Ca_3(PO_4)_2 \cdot CaCO_3 \cdot 6H_2O$. The comparatively low solubility of bone ash in citrate solution may be due to the formation of oxyapatite upon ignition of the carbonato-phosphate.

$$3Ca_3(PO_4)_2 \cdot CaCO_3 = 3Ca_3(PO_4)_2 \cdot CaO + CO_2$$

PHOSPHATE ROCK

The effect of the weight of sample on the percentages of citrate-insoluble phosphoric acid in 13 samples of mineral calcium phosphates and 1 sample of Peruvian guano was determined. The samples include commercial material from the Florida land-pebble and hard-rock phosphate deposits, the Tennessee brown- and blue-rock phosphate deposits, and the Idaho and Wyoming phosphate deposits. Analyses were also made on samples of fluorapatite from Quebec, commercial phosphate rock from Curaçao, Dutch West Indies, Florida soft and waste-pond phosphates, and colloidal material extracted by means of the supercentrifuge from Tennessee brown-rock phosphate and Florida soft and waste-pond phosphates. With one exception, that of the Peruvian guano, which was ground to 20 mesh, all samples were ground to pass a 100-mesh sieve. The chemical and physical composition of the samples of colloidal phosphates and the Florida soft and waste-pond phosphates used in this study have been discussed by Jacob, Hill and Holmes (12) and Hill, Jacob, Alexander and Marshall (8).

The figures given in Table 4 show that none of the types of phosphate rock occurring in the United States contains significant quantities of citrate-soluble phosphoric acid as determined by the official method, and the percentage of citrate-insoluble phosphoric acid in these materials does not decrease to an important extent when the weight of sample is reduced from 2.0 to 0.5 gram. The phosphoric acid in Peruvian guano is completely soluble in neutral ammonium citrate solution when less than 2.0 grams is taken for analysis. The results show that the phosphoric acid in the colloidal material extracted from the Florida soft and waste-pond phosphates is no more soluble in citrate solution than the phosphoric acid in the original materials. On the other hand, the weight of phosphoric acid dissolved from the colloidal material extracted from Tennessee brown-rock phosphate was approximately twice that dissolved from the original material.

It is interesting to note that the fluorapatite was the least soluble and the Curaçao phosphate the most soluble of the samples of mineral phosphate examined. The apatite contained 3.26 per cent of fluorine, and the Curaçao phosphate contained only 0.70 per cent. Jacob and Reynolds (11) and Reynolds, Jacob and Hill (18) have shown that all the commercial types of phosphate rock now produced in the United States contain 3–4 per cent of fluorine and the Florida waste-pond phosphates contain approximately 1–2 per cent of fluorine. A further study of phosphate rock may show that there is a relationship between the content of fluorine-bearing phosphate in the rock and its solubility in neutral ammonium citrate solution.

A comparison of the figures given in Tables 2, 3 and 4 shows that the phosphoric acid in tricalcium phosphate, raw bone, steamed bone and

TABLE 3.

Citrate-insoluble phosphoric acid in bone products.

SAMPLE	MATERIAL	P_2O_5					PERCENTAGE OF TOTAL P_2O_5 PRESENT AS CITRATE-INSOLUBLE P_2O_5—WT. OF SAMPLE			
		TOTAL	CITRATE-INSOLUBLE—WT. OF SAMPLE							
			0.5 g.	1.0 g.	1.5 g.	2.0 g.	0.5 g.	1.0 g.	1.5 g.	2.0 g.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*				
1112	Raw Bone Meal	21.18	4.20	8.05	11.59	12.69	19.8	38.0	54.7	59.9
1027	Steamed Bone Meal	34.55	12.14	19.18	23.12	24.79	35.1	55.5	66.9	71.8
1028	Steamed Bone Meal	34.48	9.80	17.11	21.00	23.01	28.4	49.6	60.9	66.7
1123	Naphtha-Extracted Bone	26.13	7.40	11.59	13.56	14.00	28.3	44.4	51.9	53.6
1099	Naphtha-Extracted Bone (Steamed under 40 lb. pressure for 30 days)	38.47	22.04	27.98	30.49	31.99	57.3	72.7	79.3	83.2
954	Bone Ash	40.26	36.15	38.08	38.51	38.74	89.8	94.6	95.7	96.2
971	Bone Ash	39.52	34.38	36.03	36.57	36.67	87.0	91.2	92.5	92.8

TABLE 4.

Citrate-insoluble phosphoric acid in natural calcium phosphates other than bone.

SAMPLE	MATERIAL	P_2O_5*				PERCENTAGE TOTAL P_2O_5 PRESENT AS CITRATE-INSOLUBLE P_2O_5—WT. OF SAMPLE		
		TOTAL	CITRATE-INSOLUBLE—WT. OF SAMPLE					
			0.5 g.	1.0 g.	2.0 g.	0.5 g.	1.0 g.	2.0 g.
		per cent	*per cent*	*per cent*	*per cent*			
905	Fluorapatite	37.11	36.33	36.58	36.68	97.9	98.6	98.8
912	Florida land-pebble phosphate	35.37	32.33	33.48	34.60	91.4	94.7	97.8
932	Florida hard-rock phosphate	35.94	32.00	33.19	34.05	89.0	92.3	94.7
728	Florida soft phosphate	31.80	29.11	30.27	30.30	91.5	95.2	95.3
936	Colloidal material extracted from No. 728	31.43	28.12	29.58	30.75	89.5	94.1	97.8
726	Florida waste-pond phosphate	23.48	19.94	21.55	22.51	84.9	91.8	95.9
934	Colloidal material extracted from No. 726	18.12	15.47	16.58	17.25	85.4	91.5	95.2
762	Tennessee brown-rock phosphate	33.73	31.35	31.89	32.54	92.9	94.5	96.5
938	Colloidal material extracted from No. 762	25.02	20.37	21.74	22.91	81.4	86.9	91.6
930	Tennessee blue-rock phosphate	30.95	27.37	28.73	29.71	88.4	92.8	96.0
948	Wyoming phosphate	30.19	28.70	29.32	29.86	95.1	97.1	98.9
973	Idaho phosphate	32.83	28.82	30.17	30.98	87.8	91.9	94.4
985	Curaçao phosphate	38.59	31.60	33.80	35.67	81.9	87.6	92.4
1106	Peruvian guano†	14.40	0.00	0.00	0.40	0.0	0.0	2.8

* Results calculated to moisture-free basis (105°C) except in the case of No. 1106.
† 20-mesh material. Other samples ground to 100 mesh.

naphtha-extracted bone is approximately 5–10 times as soluble in citrate solution as is the phosphoric acid in phosphate rock. Many chemists seem to have the impression, however, that phosphate rock is essentially tricalcium phosphate. Recently, this impression has caused considerable confusion, particularly as regards the chemical nature of the tricalcium phosphate formed when superphosphate is treated with ammonia. Recent x-ray investigations indicate that the chemical constitution of the various domestic types of phosphate rock is essentially the same as that of crystalline fluorapatite, to which the empirical formula $3Ca_3(PO_4)_2 \cdot CaF_2$ is ascribed. Tricalcium phosphate has the empirical formula $Ca_3(PO_4)_2$. It will be noted that the empirical formula for apatite differs from that for bone, $3Ca_3(PO_4)_2 \cdot CaCO_3$, only in that the carbonate radical in the latter is replaced by fluorine.

ACIDULATED PHOSPHATES

The effect of the weight of sample taken for analysis on the percentages of citrate-insoluble phosphoric acid in 15 samples of acidulated phosphates was determined. The sources of these materials, their method of preparation, etc., are described briefly as follows:

Superphosphates.—The ordinary superphosphates, Nos. 1037 and 1066, were well cured commercial materials manufactured in the usual manner. Florida pebble superphosphate, No. 1060, was a commercial material prepared by a special patented process and sold under the trade name, "Oberphos." The ammoniated superphosphates, Nos. 1036, 1067 and 1050, were prepared experimentally from the above mentioned superphosphates, respectively, and contained 5.76, 4.35 and 4.97 per cent of ammonia, respectively.

The "den" superphosphates, Nos. 1073 and 1087, were obtained by artificially drying freshly manufactured commercial superphosphate in order to prevent further conversion of citrate-insoluble phosphoric acid into soluble forms. The limed superphosphate, No. 1103, was a commercial material prepared by mixing 375 pounds of ground dolomitic limestone with 1625 pounds of superphosphate. The sample was taken about one year after the mixture was prepared. With the exception of the limed superphosphate, which was ground to 100 mesh, the above mentioned materials were ground to pass a 20-mesh sieve.

Triple Superphosphates.—Triple superphosphates, Nos. 1059 and 1061, were commercial materials. Ammoniated triple superphosphate, No. 1039, contained 7.51 per cent ammonia. It was prepared experimentally from Tennessee brown-rock triple superphosphate, but the material was not the same as that from which sample No. 1059 was taken. These samples were ground to 20 mesh.

Wet-Mixed Base Goods.—These materials, Nos. 1104 and 1105, were commercial products prepared by treating mixtures of phosphate rock and

nitrogenous organic materials, such as fur waste, calcium cyanamide, leather, etc., with sulfuric acid. The samples were ground to 20 mesh.

"Reform" Phosphate.—This material, No. 1109, was a commercial product manufactured (14), (17) in Europe by treating phosphate rock with a limited quantity of sulfuric acid.

The figures given in Table 5 show that decreasing the ratio of weight of sample to volume of citrate solution does not bring about a considerable decrease in the percentages of citrate-insoluble phosphoric acid in non-ammoniated superphosphates and triple superphosphates, "den" superphosphate, limed superphosphate, wet-mixed base goods and "reform" phosphate. In the case of heavily ammoniated ordinary superphosphate, however, there is a progressive and significant decrease in the percentage of citrate-insoluble phosphoric acid when the weight of sample is decreased by 0.5 gram steps from 2.0 to 0.5 gram. This is due principally to the presence in these materials of the difficultly soluble tricalcium phosphate. Decreasing the weight of sample did not greatly affect the percentage of citrate-insoluble phosphoric acid in the sample of ammoniated triple superphosphate, No. 1039. This was due to the fact that the water-insoluble phosphoric acid in this particular sample was present principally in the form of dicalcium phosphate, which, comparatively speaking, is readily soluble in citrate solution. The nature of the phosphate compounds in the citrate-insoluble residues from superphosphates and ammoniated superphosphates has been discussed by Jacob, Hill, Ross and Rader (13).

The results given in Table 5 show that, except in the case of heavily ammoniated ordinary superphosphate and "reform" phosphate, the weight of phosphoric acid dissolved by citrate solution from the acidulated phosphates was roughly proportional to the weight of sample. On the other hand, as the weight of sample was increased beyond 0.5 gram, the weight of phosphoric acid dissolved from heavily ammoniated ordinary superphosphate and "reform" phosphate did not increase proportionately, but it rapidly approached the maximum quantity that can be dissolved from these materials under the conditions of the determinations.

BASIC SLAG AND CALCINED PHOSPHATE

The relation between the weight of sample and the quantity of phosphoric acid dissolved by 100 cc. of citrate solution was determined on 2 samples of basic slag and 4 samples of calcined phosphates. The results are given in Table 6.

The samples of basic slag, Nos. 1107 and 1108, were high-grade commercial materials manufactured in Europe. Fluorspar was not used in the production of these slags. The samples of calcined phosphate, Nos. 1043, 1044 and 1111, were prepared experimentally. As described by Guernsey and Yee (4), this material is made by heating to a temperature of approxi-

TABLE 5.

Citrate-insoluble phosphoric acid in acidulated phosphates.

SAMPLE	MATERIAL	P_2O_5					
		TOTAL	WATER-INSOLUBLE	CITRATE-INSOLUBLE—WT. OF SAMPLE			
				0.5 g.	1.0 g.	1.5 g.	2.0 g.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1037	Florida pebble superphosphate	18.90	1.71	0.28	0.25	0.28	0.27
1060	Florida pebble superphosphate	20.59	3.48	0.96	1.08	1.10	1.14
1066	Tennessee brown-rock superphosphate	19.41	5.22	0.22	0.19	0.35	0.28
1036	Ammoniated Florida pebble superphosphate	18.67	11.03	1.02	1.70	2.67	3.66
1050	Ammoniated Florida pebble superphosphate	19.54	13.67	2.95	5.01	6.24	7.15
1067	Ammoniated Tennessee brown-rock superphosphate	19.25	11.38	0.33	1.11	2.55	3.76
1103	Limed Florida pebble superphosphate	15.26	14.63	1.29	1.50	1.79	1.70
1073	Florida pebble "den" superphosphate	22.22	17.76	3.19	3.35	3.35	3.41
1087	Tennessee brown-rock "den" superphosphate	21.97	5.66	1.21	1.30	1.33	1.42
1061	Idaho triple superphosphate	45.58	8.58	3.51	3.80	3.93	3.80
1059	Tennessee brown-rock triple superphosphate	44.07	11.18	1.56	1.58	1.53	1.47
1039	Ammoniated Tennessee brown-rock triple superphosphate	44.96	25.41	1.11	1.41	1.61	1.88
1104	Wet-mixed base goods	15.08	11.22	1.08	1.21	1.28	1.26
1105	Wet-mixed base goods	11.36	5.89	2.77	2.89	2.94	3.13
1109	"Reform" phosphate	26.06	23.88	17.05	19.42	19.84	20.41

TABLE 6.

Citrate-insoluble phosphoric acid in basic slags and calcined phosphates.

SAMPLE	MATERIAL	P_2O_5					PERCENTAGE OF TOTAL P_2O_5 PRESENT AS CITRATE-INSOLUBLE P_2O_5—WT. OF SAMPLE			
		TOTAL	CITRATE-INSOLUBLE—WT. OF SAMPLE							
			0.5 g.	1.0 g.	1.5 g.	2.0 g.	0.5 g.	1.0 g.	1.5 g.	2.0 g.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*				
1107	Basic slag	23.36	2.21	5.73	9.48	11.33	9.5	24.5	40.6	48.5
1108	Basic slag	19.07	2.77	7.04	9.91	11.94	14.5	36.9	52.0	62.6
1043	Calcined phosphate	33.20	8.14	8.73	9.92	11.85	24.5	26.3	29.9	35.7
1044	Calcined phosphate	33.75	1.80	1.80	1.97	4.91	5.3	5.3	5.8	14.5
1111	Calcined phosphate	26.57	9.00	9.53	10.99	12.10	33.9	35.9	41.4	45.5
1110	"Non-acid" phosphate	27.48	10.43	11.49	13.93	16.60	38.0	41.8	50.7	60.4

mately 1300°C. a mixture of ground phosphate rock, an alkali salt, and carbon or a carbonaceous material. The sample of "non-acid" phosphate, No 1110, was a commercial material manufactured at Lakeland, Fla., in 1925 by the Kreiss process (1), which involves the heating of a slurry of crushed phosphate rock and a potash salt to clinkering in a rotary kiln.

The figures given in Table 6 show that in general there is a progressive and significant decrease in the percentage of citrate-insoluble phosphoric acid in these materials, particularly basic slag, when the weight of sample is decreased by 0.5 gram steps from 2.0 to 0.5 gram.

The figures obtained on basic slag are of particular interest because they indicate that by decreasing the weight of sample it may be possible to obtain satisfactory results for available phosphoric acid in these materials by the use of neutral ammonium citrate solution instead of the customary 2 per cent citric acid solution. In the case of slag, No. 1107, 25.7 per cent of the total phosphoric acid was insoluble in 2 per cent citric acid as determined by the official method for the evaluation of phosphoric acid in basic slag. This figure is very close to the result, 24.5 per cent of the total phosphoric acid as insoluble phosphoric acid, obtained when a 1.0 gram sample of this slag was digested for 30 minutes with 100 cc. of citrate solution. In the case of slag No. 1108, 18.4 per cent of the total phosphoric acid was insoluble in 2 per cent citric acid solution. This figure lies between the results, 14.5 and 36.9 per cent, obtained by digesting 0.5 and 1.0 gram samples, respectively, with citrate solution. A single method for the laboratory determination of the availability of all phosphatic fertilizer materials would be desirable, and in this connection further studies on the relative solubility of basic slag in neutral ammonium citrate and 2 per cent citric acid solutions are planned.

MAGNESIUM PHOSPHATES

The solubilities of di- and trimagnesium phosphates, magnesium ammonium phosphate and magnesium pyrophosphate in neutral ammonium citrate solution were determined. The content of total phosphoric acid and magnesium oxide and the phosphoric acid-magnesium oxide ratios in these samples are given in Table 7. Their source, method of preparation, etc., are described briefly as follows:

Dimagnesium Phosphate.—This sample, No. 1096, was Kahlbaum's C. P. crystalline material.

Magnesium Ammonium Phosphate.—This sample, No. 1118, was prepared in the laboratory by precipitating an aqueous solution of pure phosphoric acid with ammoniacal magnesium chloride solution. The precipitate was dried for several weeks over concentrated sulfuric acid.

Trimagnesium Phosphate.—This sample, No. 1122, was prepared by slowly adding a solution of trisodium phosphate to a solution containing an excess of magnesium sulfate. The precipitate was washed with cold

water until the filtrate gave no test for sulfates, and was dried at a temperature of approximately 75°C.

Magnesium Pyrophosphate.—This sample, No. 1097, was composed of residues obtained by the ignition of magnesium ammonium phosphate in the gravimetric determination of phosphoric acid.

The figures given in Table 8 show that dimagnesium phosphate and magnesium ammonium phosphate are completely soluble in citrate solution when 2.0 gram samples are taken for analysis. Trimagnesium phosphate is completely soluble when 1.0 gram samples are used, but when the weights of sample are increased to 1.5 and 2.0 grams, 3.3 and 29.3 per cent, respectively, of the total phosphoric acid is insoluble. Magnesium pyrophosphate prepared by igniting magnesium ammonium phosphate is

TABLE 7.
Composition of magnesium phosphates.

SAMPLE	MATERIAL	P_2O_5	MgO	P_2O_5, MgO RATIO
		per cent	*per cent*	
1096	$MgHPO_4$	40.31	23.31	1.729*
1118	$MgNH_4PO_4$	29.47	16.48	1.788*
1122	$Mg_3(PO_4)_2$	39.28	33.64	1.168†
1097	$Mg_2P_2O_7$	63.95	36.20	1.767*

* Theoretical P_2O_5–MgO ratio = 1.762.
† Theoretical P_2O_5–MgO ratio = 1.175.

practically insoluble in citrate solution. Comparison of the figures given in Tables 2 and 8 shows that the di- and trimagnesium phosphates are much more soluble in citrate solution than the corresponding calcium salts.

IRON AND ALUMINUM PHOSPHATES

The percentages of total phosphoric acid, aluminum oxide and ferric oxide and the ratios of phosphoric acid to aluminum oxide and ferric oxide in the samples of iron and aluminum phosphates used in this investigation are given in Table 9. The source of the materials, their method of preparation, etc., are described briefly as follows:

Aluminum Phosphates.—Samples Nos. 843 and 854 were Coleman and Bell's and Baker and Adamson's C. P. materials, respectively. The phosphoric acid-aluminum oxides ratios in these materials show that they contained more phosphoric acid than is theoretically required for pure secondary aluminum phosphate, $Al_2(HPO_4)_3$.

Iron Phosphates.—Samples Nos. 1034 and 1035 were Eimer and Amend's and Coleman and Bell's C. P. materials, respectively. The phosphoric acid-ferric oxide ratios indicate that sample No. 1034 was a mixture of secondary and tertiary ferric phosphates, and that sample No. 1035 was essentially tertiary ferric phosphate.

TABLE 8.

Citrate-insoluble phosphoric acid in magnesium phosphates.

SAMPLE	MATERIAL	P_2O_5					PERCENTAGE OF TOTAL P_2O_5 PRESENT AS CITRATE-INSOLUBLE P_2O_5—WT. OF SAMPLE			
		TOTAL	CITRATE-INSOLUBLE—WT. OF SAMPLE							
			0.5 g.	1.0 g.	1.5 g.	2.0 g.	0.5 g.	1.0 g.	1.5 g.	2.0 g.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*				
1096	$MgHPO_4$	40.31	0.00	0.00	0.00	0.00	0.0	0.0	0.0	0.0
1118	$MgNH_4PO_4$	29.47	0.00	0.00	0.00	0.00	0.0	0.0	0.0	0.0
1122	$Mg_3(PO_4)_2$	39.28	0.00	0.00	1.28	11.50	0.0	0.0	3.3	29.3
1097	$Mg_2P_2O_7$	63.95	61.12	61.27	—	62.61	95.6	95.8	—	97.9

TABLE 9.
Composition of iron and aluminum phosphates.

SAMPLE	MATERIAL	P_2O_5	Fe_2O_3	Al_2O_3	P_2O_5–Fe_2O_3 RATIO*	P_2O_5–Al_2O_3 RATIO†
		per cent	*per cent*	*per cent*		
843	Aluminum phosphate	28.27	—	12.46	—	2.269
854	Aluminum phosphate	43.12	—	19.29	—	2.235
1034	Iron phosphate	37.46	32.56	—	1.150	—
1035	Iron phosphate	20.75	24.64	—	0.842	—
829	Insoluble material precipitated by Na_2CO_3 from Tennessee brown-rock phosphoric acid‡	29.54	8.32	8.64	—	—
1101	Insoluble material precipitated by NH_3 from Idaho phosphoric acid	43.78	4.10	17.30	—	—
1102	Insoluble material precipitated by NH_3 from Tennessee brown-rock phosphoric acid	40.16	8.80	15.30	—	—
904	Natural hydrated Fe and Al phosphate from Connetable Islands	42.21	11.00§	29.00§	—	—

* Theoretical P_2O_5–Fe_2O_3 ratios in $FePO_4$ and $Fe_2(HPO_4)_3$ = 0.890 and 1.335, respectively.
† Theoretical P_2O_5–Al_2O_3 ratios in $AlPO_4$ and $Al_2(HPO_4)_3$ = 1.393 and 2.089, respectively.
‡ Also contained 8.50 per cent CaO.
§ Approximate percentages.

TABLE 10.

Citrate-insoluble phosphoric acid in iron and aluminum phosphates.

SAMPLE	MATERIAL	P_2O_5					PERCENTAGE OF TOTAL P_2O_5 PRESENT AS CITRATE-INSOLUBLE P_2O_5—WT. OF SAMPLE			
		TOTAL	CITRATE-INSOLUBLE—WT. OF SAMPLE							
			0.5 g.	1.0 g.	1.5 g.	2.0 g	0.5 g.	1.0 g.	1.5 g	2.0 g.
		per cent	*per cent*	*per cent*	*per cent*	*per cent*				
843	Aluminum phosphate	28.27*	0.44	0.34	0.34	0.28	1.6	1.2	1.2	1.0
854	Aluminum phosphate	43.12†	16.20	20.28	19.79	23.93	37.6	47.0	45.9	55.5
1034	Iron phosphate	37.46‡	6.37	6.68	6.70	6.80	17.0	17.8	17.9	18.2
1035	Iron phosphate	20.75§	2.94	2.94	3.06	3.06	14.2	14.2	14.7	14.7
829	Insoluble material precipitated by Na_2CO_3 from Tennessee brown-rock phosphoric acid	29.54**	1.63	1.65	1.67	1.94	5.5	5.6	5.7	6.6
1101	Insoluble material precipitated by NH_3 from Idaho phosphoric acid	43.78	0.00	0.00	0.00	0.00	0.0	0.0	0.0	0.0
1102	Insoluble material precipitated by NH_3 from Tennessee brown-rock phosphoric acid	40.16	0.00	0.00	0.08	0.19	0.0	0.0	0.2	0.5
904	Natural hydrated Fe and Al phosphate from Connetable Islands	42.21	38.24	38.91	—	39.61	90.6	92.2	—	93.8

* Water-soluble P_2O_5=10.44 per cent.
† Water-soluble P_2O_5= 0.20 per cent.
‡ Water-soluble P_2O_5= 1.12 per cent.
§ No water-soluble P_2O_5.
** Water-soluble P_2O_5=2.26 per cent.

Sample No. 829 was material precipitated from crude phosphoric acid by sodium carbonate. This material is obtained as a by-product of the commercial manufacture of di- and trisodium phosphates and is sold to the fertilizer trade. Samples Nos. 1101 and 1102 were materials precipitated by neutralizing crude phosphoric acid with ammonia. The precipitated material was washed with cold water and dried at 110°C. Sample No. 904 was a natural hydrated iron and aluminum phosphate from the Connetable Islands, off the coast of French Guiana.

The figures given in Table 10 show that decreasing the weight of sample from 2.0 to 0.5 gram does not in general bring about a significant decrease in the percentage of citrate-insoluble phosphoric acid in iron and aluminum phosphates. The samples of supposedly pure materials used in this investigation differed considerably in their solubility in citrate solution, the percentages of total phosphoric acid present as citrate-insoluble phosphoric acid ranging from 1.0 to 55.5 when 2.0 gram samples of the materials were taken for analysis. The impure materials precipitated from crude phosphoric acid by sodium carbonate and ammonia are almost completely soluble in citrate solution, while natural iron and aluminum phosphates are practically insoluble. A further study of the citrate solubility of pure iron and aluminum phosphates in which the salts of known chemical constitution are used is desirable.

ACKNOWLEDGMENT

The writers are indebted to W. L. Hill and H. L. Marshall for the aluminum, calcium, iron and magnesium determinations given in this paper.

SUMMARY

A study was made of the solubility of 64 samples of various phosphatic materials in neutral ammonium citrate solution as affected by the weight of sample taken for analysis.

The materials investigated included di- and tricalcium phosphates, magnesium, iron and aluminum phosphates, various bone products, acidulated and non-acidulated natural phosphates, basic slags and calcined phosphates.

When the weight of sample was decreased by 0.5 gram steps from 2.0 to 0.5 gram there was a progressive and significant decrease in the percentage of citrate-insoluble phosphoric acid in di- and tricalcium phosphates, trimagnesium phosphate, heavily ammoniated superphosphates, raw, steamed and naphtha-extracted bone, basic slag and calcined phosphate.

Dimagnesium phosphate and magnesium ammonium phosphate were completely soluble in citrate solution when 2.0 gram samples were used.

Pure dicalcium phosphate and trimagnesium phosphate were completely soluble in citrate solution when 1.0 gram samples were used, but

both of these materials contained small quantities of citrate-insoluble phosphoric acid when the weight of sample was increased to 1.5–2.0 grams.

Calcium and magnesium pyrophosphates prepared by igniting dicalcium phosphate and magnesium ammonium phosphate, respectively, were only slightly soluble in citrate solution.

Calcium hydroxyphosphate was approximately one-third as soluble as tricalcium phosphate in citrate solution.

Approximately 65–75 per cent of the total phosphoric acid in tricalcium phosphate was insoluble in citrate solution when 2.0 gram samples were used, but when the weight of sample was reduced to 0.5 gram only about 18–36 per cent of the total phosphoric acid was insoluble.

Under similar conditions, the solubility of the phosphoric acid in raw, steamed and naphtha-extracted bone approximated closely the solubility of the phosphoric acid in tricalcium phosphate.

The samples of C. P. iron and aluminum phosphates used in this investigation contained 1.0–55.5 per cent of the total phosphoric acid in the form of citrate-insoluble phosphoric acid when 2.0 gram samples were used, and significant decreases in the percentages of citrate-insoluble phosphoric acid were not obtained by decreasing the weight of sample.

Impure iron and aluminum phosphates precipitated from crude phosphoric acid by sodium carbonate and by ammonia were almost completely soluble in citrate solution.

Decreasing the weight of sample from 2.0 to 0.5 gram did not bring about a significant decrease in the percentages of citrate-insoluble phosphoric acid in superphosphate and other acidulated phosphates that had not been treated with ammonia, or otherwise reverted.

There was a progressive and significant decrease in the percentage of citrate-insoluble phosphoric acid in highly ammoniated superphosphate when the weight of sample was decreased in 0.5 gram steps from 2.0 to 0.5 gram.

Less than 10 per cent of the total phosphoric acid present in commercial types of phosphate rock occurring in the United States was soluble in citrate solution when 2.0 gram samples were taken for analysis, and a significant decrease in the percentage of citrate-insoluble phosphoric acid in such materials was not obtained by decreasing the weight of sample to 0.5 gram.

A progressive and significant decrease in the percentage of citrate-insoluble phosphoric acid in high-grade, fluorine-free basic slag was obtained by decreasing the weight of sample from 2.0 to 0.5 gram in 0.5 gram steps. When 0.5–1.0 gram samples of high-grade basic slag were treated with 100 cc. of neutral ammonium citrate solution, approximately the same percentage of the total phosphoric acid was dissolved as when the samples were treated with 2 per cent citric acid solution according to the official method.

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DETERMINATION OF CARBON DIOXIDE IN SOIL CARBONATES—A MODIFICATION OF THE OFFICIAL METHOD[1]

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In previous studies by the present general referee and L. G. Willis[2] of this laboratory it was pointed out that considerable quantities of carbon dioxide are evolved when an acid, carbonate-free soil is boiled with hydrochloric acid. It was further determined that this carbon dioxide which comes from the soil organic matter was reduced to a minimum and that a complete evolution and recovery of carbon dioxide from calcareous materials was obtained when 50-gram charges of soil were agitated and aspirated with hydrochloric acid (1+9) in the cold for 30 minutes. In the case of certain soils of the glaciated region and those to which dolomite additions had been made, it was found that a longer period of agitation and aspiration was required. In the earlier work neither the exact number of aspiration bubbles nor the aspiration volume was given. In the present investigation these two points were considered in studying the rate of aspiration that is essential to the complete recovery of evolved carbon di-

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1930.
[2] *J. Ind. Eng. Chem.*, **7**, 227 (1915).

oxide when the complete disintegration of carbonates is effected within the 30-minute period of agitation at room temperature. The use of reducing materials that would permit the heating required to decompose quickly the more resistant natural carbonates with a minimum interference from organic matter was also considered.

RATE AND PERIOD OF ASPIRATION

The complete removal of the liberated carbon dioxide and its collection in the absorption apparatus is influenced by the time, size, and shape of evolution flask, dimensions and complexity of purifying train, mode of contact between solid-liquid-gas phases and the rate of air passage through the apparatus.

The official method[1] prescribes a 300 ml. Erlenmeyer evolution flask. Between this flask and the absorption tower a spherical bulb of about 50 ml. capacity is inserted. The liberation is effected at room temperature, with constant agitation on a shaking machine. This equipment was used to study the rate of aspiration, which is not specified by the official procedure. Replicate charges of precipitated calcium carbonate were weighed into evolution flasks and run according to the official procedure, except that the carbon dioxide was determined at successive 30-minute intervals and with the specific aspiration rates of 4 and 6 liters per hour.

The data of Table 1 demonstrate that practically all the carbon dioxide was removed from the evolution flash when aspiration was carried out for 30 minutes after complete decomposition of the sample if the rate of flow was 6 liters per hour, but that at a rate of only 4 liters per hour the carbon dioxide recovery fell short. The recovery of carbon dioxide from calcium carbonate was 0.5 per cent less than the recovery by the gravimetric determination when ascarite was used as the absorbent. When the sodium hydroxide absorption towers were supplemented by flasks containing calcium hydroxide, it was found that some carbon dioxide had passed through the sodium hydroxide when the rate was 6 liters per hour. It, therefore, appears that when high-carbonate materials are used the 6-liter-per-hour rate may be too great to permit the complete absorption of the carbon dioxide by the sodium hydroxide in the tall bead-filled tower. In an attempt to obviate this difficulty an ascarite absorption bulb was substituted for the sodium hydroxide solution. The high absorptive capacity of ascarite has been well established by Hillebrand and Lundell,[2] and this absorbent is admirably adapted to the stoppage of carbon dioxide at high rates of flow. The gravimetric determination of the carbon dioxide necessitates a train of desiccants. This insertion can be provided, however, without great inconvenience, as will be shown later.

[1] *Methods of Analysis*, A.O.A.C., 1925, 24.
[2] "Applied Inorganic Analysis," 1st ed., p. 42.

DISINTEGRATION OF DOLOMITIC LIMESTONE BY HYDROCHLORIC ACID (1+9)

The slow disintegration of dolomitic rocks with hydrochloric acid (1+9) at room temperature introduces another factor. A comparison of the results of Tables 1 and 2 shows that, under the given conditions, a 100-mesh dolomitic rock will require at least 1/2 hour longer for its complete solution than would a 100-mesh calcite. With soils containing dolomite it may become necessary to reduce large quantities to a 100-mesh fineness. This process involves considerable labor when a large run of carbon dioxide determinations is required, even when a grinding machine is available. Furthermore, samples of 100–200 grams cannot be ground on the

TABLE 1.

Effect of rate of aspiration at room temperature upon the recovery of CO_2 from $CaCO_3$ from 1 gram charge treated with 60 cc. of HCl (1+9)

SUCCESSIVE PERIODS	VOLUME ASPIRATED PER HOUR	
	4 liters	6 liters
	gram	*gram*
1st, 30 minutes	0.4130	0.4307
2nd, 30 minutes	0.0171	0.0011
Total, 1 hour	0.4301	0.4318

TABLE 2.

CO_2 recoveries from dolomites of different fineness by agitation with HCl (1+9) at room temperature and aspiration of 6 liters per hour

SUCCESSIVE PERIODS	40-60 mesh*	60-100 mesh*	100-200 mesh†
	gram	*gram*	*gram*
1st, 30 minutes	0.3240	0.3538	0.4205
2nd, 30 minutes	0.0625	0.0630	0.0404
3rd, 30 minutes	0.0335	0.0075	0.0000
4th, 30 minutes	0.0025	0.0020	0.0000
Total, 2 hours	0.4225	0.4263	0.4609
At boiling heat		0.4260	0.4715

* Absorption by ascarite.
† Absorption by NaOH solution.

machine without a high percentage of waste and danger of the segregation of minerals in the waste. On the other hand 40–50-mesh samples of dolomite require a longer period to insure disintegration with hydrochloric acid (1+9) as may be seen from the data of Table 2. The carbon dioxide determinations from the 1-gram charges of the 40–60- and 60–100-mesh dolomite were made gravimetrically by absorption in ascarite, whereas the 100–200-mesh determination was made volumetrically by the procedure previously described. The data for the 100–200-mesh dolomite show complete removal of carbon dioxide with 1 hour's aspiration, but with in-

complete absorption, probably due to high rate of carbon dioxide passage through the sodium hydroxide solution. The 60–100-mesh sample gave a complete recovery of carbon dioxide with 2 hours of aspiration. The recovery from the 40–60-mesh dolomite was not so rapid, but it was also complete within 2 hours. If the 60–100-mesh is taken as representing a workable fineness for carbonate determinations, it may be concluded that 2 hours' aspiration is necessary for the determination of carbon dioxide with agitation in hydrochloric acid (1+9) at room temperature. Clark and Collins[1] state that a 5-hour period is required for the complete recovery of carbon dioxide from calcite. It is possible that their stirring device is not so efficient as the shaking device of MacIntire and Willis.[2]

INFLUENCE OF SOIL ORGANIC MATTER ON CARBONATE-CARBON DIOXIDE RESULTS

The vitiating influence of organic matter on the determination of carbon dioxide in carbonates at boiling heat was pointed out as early as 1890 by Van Bemmelen.[3] This factor was also considered by Marr,[4] who suggested the use of very dilute hydrochloric acid (1+50) with boiling under reduced pressure at 50°C. for 20 minutes. MacIntire and Willis[2] sought to eliminate this source of error by a method that effects the decomposition of carbonates at room temperature. Their method is essentially the present official method for carbon dioxide for soils. Careful and repeated observations have shown, however, that appreciable quantities of carbon dioxide may be given off from some carbonate-free soils, at room temperatures, when in contact with dilute hydrochloric acid (1+9). The quantities evolved during short intervals are generally small, but when the contact period is extended over 1½ or 2 hours the resultant error may be appreciable with some soils.

The data in Table 3 show the error incurred from soils of varying organic matter content when agitated with hydrochloric acid alone. When the manipulations were carried out without soil for a period of 2 hours, plus errors of 1 to 2 mg. were encountered. Since these soils were known to be devoid of carbonates, it is evident that erroneous conclusions may be drawn as to an apparent carbonate content on the basis of 2-hour yields of carbon dioxide. An attempt, therefore, was made to eliminate the action of the interference of organic matter by the addition of reducing compounds to the liberating acid.

USE OF REDUCING MATERIALS TO COUNTERACT ORGANIC MATTER DECOMPOSITION

The continuous evolution of carbon dioxide during long periods of agitation of a soil suspension in hydrochloric acid (1+9) did not appear to be correlated with high organic matter content. In a soil with a high iron-

[1] *Soil Sci.*, 27, 407 (1929).
[2] *Loc. cit.*
[3] *Landw. Vers. Sta.*, 37, 279 (1890).
[4] *J. Agr. Sci.*, 3, 155 (1908).

manganese content and a fair supply of organic matter there seemed to be a greater outgo of carbon dioxide derived from organic matter than was found for soils high in organic matter and of low iron-manganese content. It was assumed that the oxidation was due to the formation of nascent chlorine through reaction of the hydrochloric acid upon manganese compounds. The oxidation of organic matter by ferric chloride was also considered as a possibility.

TABLE 3.

Sustained evolution of CO_2 from 50 gram charges of carbonate-free soils agitated with HCl (1+9) at room temperature

SOIL	CO_2 EVOLVED			$CaCO_3$ EQUIVALENCE OF TOTAL CO_2	
	1st hour	2nd hour	2-hour total		
	gram	*gram*	*gram*	*per cent*	*lbs. per 2 million lbs. of soil*
Cumberland loam	0.0080	0.0075	0.0155	0.070	1,400
Crossville loam	0.0025	0.0030	0.0055	0.025	500
Grass sod	0.0250	0.0095	0.0345	0.157	3,140
Sandy loam	0.0018	0.0000	0.0018	0.004	80

TABLE 4.

Effect of ferrous chloride and of stannous chloride on the evolution of CO_2 from soil organic matter on agitation at room temperature with HCl (1+9)

SOIL	CO_2 EVOLVED FROM 50 GRAM CHARGES OF SOIL DURING 1 HOUR		
	HCl only	HCl+$FeCl_3$	HCl+$SnCl_2$
	per cent	*per cent*	*per cent*
Cumberland loam No. 6355	0.0130	0.0020	0.0015
Crossville loam No. 6348	0.0060		0.0015
Jackson silt loam No. 6358	0.0190	0.0140	0.0130
"Virginia"	0.0130		0.0090
"Virginia"	0.0265		0.0185
Grass sod	0.0270	0.0040	0.0030

After a study of this phase of the work had begun, there appeared a preliminary paper by Schollenberger,[1] who proposed the use of ferrous chloride as an antioxidant in the determination of carbon dioxide in soils. His findings as to the oxidative effect of manganese oxides of the soil are in accord with those of the writer. In certain instances, however, it appeared that ferric chloride caused greater oxidation of soil organic matter than did manganese dioxide. Stannous chloride was therefore used in comparison with ferrous chloride.

The comparative effects of these two chemicals in suppressing the yields of carbon dioxide from organic matter in contact with hydrochloric acid (1+9) at room temperature may be seen from the data of Table 4. Sam-

[1] *Science*, 72, 13 (1930).

ples 1, 2 and 3, known to have been unlimed, gave practically no carbon dioxide when stannous chloride was introduced into the hydrochloric acid solution. The two sandy soils, 4 and 5, had received additions of lime and dolomite, respectively, 4 years previous to sampling and apparently contained small residues of the added carbonates. Sample 3 yields an alkaline reaction in water suspensions and probably contains small quantities of natural calcium carbonate. When the blank is subtracted, it is evident that both ferrous chloride and stannous chloride completely eliminated the vitiating influence of organic matter on carbonate-carbon dioxide results during the agitation with hydrochloric acid at room temperature.

EFFECT OF REDUCING CHEMICALS ON SOIL ORGANIC MATTER IN HYDROCHLORIC ACID (1+9) AT BOILING TEMPERATURE

If the procedure is to be effective for soils without exception, it should give equally accurate results for both dolomitic and calcareous limestones. The technic thus far developed would require a 2 hour aspiration period as a safe minimum for soils containing considerable dolomitic limestone. Schollenberger[1] has recently proposed a procedure by which the

TABLE 5.

Effect of ferrous chloride and of stannous chloride on the evolution of CO_2 from soil organic matter on agitation at boiling temperature with HCl (1+9)

SOIL	CO_2 EVOLVED FROM 50 GRAM CHARGES OF SOIL DURING 1 HOUR		
	HCl only	HCl+$FeCl_2$	HCl+$SnCl_2$
	per cent	*per cent*	*per cent*
Cumberland loam No. 6366	0.0445	0.0070	0.0045
Crossville	0.0220	0.0080	0.0060
Jackson	0.0235	0.0160	0.0190
"Rhode Island" loam	0.0255	0.0220	0.0110
Grass sod	0.0515	0.0230	0.0130
"Virginia" No. 123	0.0130	0.0130	0.0100
"Virginia" No. 125	0.0250	0.0250	0.0250
Ford soil	0.0410	0.0060	0.0055

soil carbonates are decomposed in dilute hydrochloric acid-ferrous chloride solution with boiling under vacuum at 28°–30°C. This procedure appears to possess a high degree of accuracy and requires simple apparatus; on the other hand, the technic is subject to the inherent difficulties connected with use of vacuum.

It was thought that the use of reducing chemicals might permit heating without appreciable effect on the soil organic matter and thus greatly shorten the time necessary for decomposition of dolomite samples. After

[1] Acknowledgment is made to Professor Schollenberger for his kindness in permitting the use of his manuscript of the paper herein referred to in advance of its publication.

a number of runs with standard dolomite samples, the procedure later to be described as the stannous chloride-hydrochloric acid method for carbonate determination was adopted.

The data of Table 5 show that both ferrous chloride and stannous chloride exerted a strong influence in suppressing the carbon dioxide evolution caused by the decomposition of organic matter upon heating. In most instances the stannous chloride proved to be superior to the ferrous chloride. The carbon dioxide yields under most favorable conditions were, however, larger than those from the corresponding samples treated at room temperature. But if the determinations at room temperature are taken as absolute it is found that the excessive carbon dioxide evolutions due to heating in four cases out of six are less than 0.01 per cent carbon dioxide; in the other two cases the value is between 0.01 and 0.02 per cent. The higher figures may be expected from samples containing a high proportion of unhumified organic matter, as in the grass sod. The stannous chloride-hydrochloric acid procedure with heat would have a decided advantage over the one at room temperature, in that it would insure the complete disintegration of the more resistant carbonates such as dolomites and magnesite in short and well-defined time periods, and with but very slight interference from organic matter. An expeditious procedure is thus afforded for the determination of carbonate carbon dioxide in soils where an error of 0.01 per cent may be tolerated. For greater accuracy, the stannous chloride-hydrochloric acid procedure should be carried out at room temperature with aspiration and agitation continued 2 hours, and with additional tests for complete decomposition of the more resistant carbonates.

STANNOUS CHLORIDE-HYDROCHLORIC ACID METHOD FOR CARBONATE DETERMINATION

APPARATUS

The complete assembly of the apparatus providing for four simultaneous determinations is shown in Figs. 1 and 2. The 300 ml. Erlenmeyer flask, the separatory funnel, and the condenser are held together as a unit on the shaker rack (*A*, Fig. 2) by means of supporting rods *E* and clamps *F*. The absorption trains of Fig. 1 are held on the shelf *J*, which extends into space equidistant with *A*. This shelf is fastened to the wall at its distant end and is braced from the permanent shelf *D*, Fig. 2. The moving parts are connected to the absorption trains by means of heavy-wall rubber tubing, sufficient slack being allowed to avoid undue strain on the glass parts during agitation. The rack *A* is suspended from the arm *C* by means of copper strips, *B*. The motor *M* has a speed control. The horizontal motion is accomplished by means of the pulley *J*, the eccentric disc *K*, and the cleft arm *L*, connected with one end of *A*, Fig. 2.

REAGENTS

(a) *Stannous chloride-hydrochloric acid solution.*—To 95 ml. of hydrochloric acid (1+9), add 5 grams of stannous chloride and heat, adding small pieces of metallic tin till the solution of the salt is complete. Cool, place in a reagent bottle, and cover the solution with a 1–2 cm. layer of mineral oil. Provide with a syphon for drawing off the solution for use.

(b) *Silver sulfate suspension.*—Fill tube *A* about ⅔ full with sulfuric acid(1+19) and add about 0.1 gram of silver sulfate.

(c) *Concentrated sulfuric acid.*

(d) *Granular calcium chloride.*

(e) *Anhydrous magnesium perchlorate.*[1]

(f) *Ascarite.*

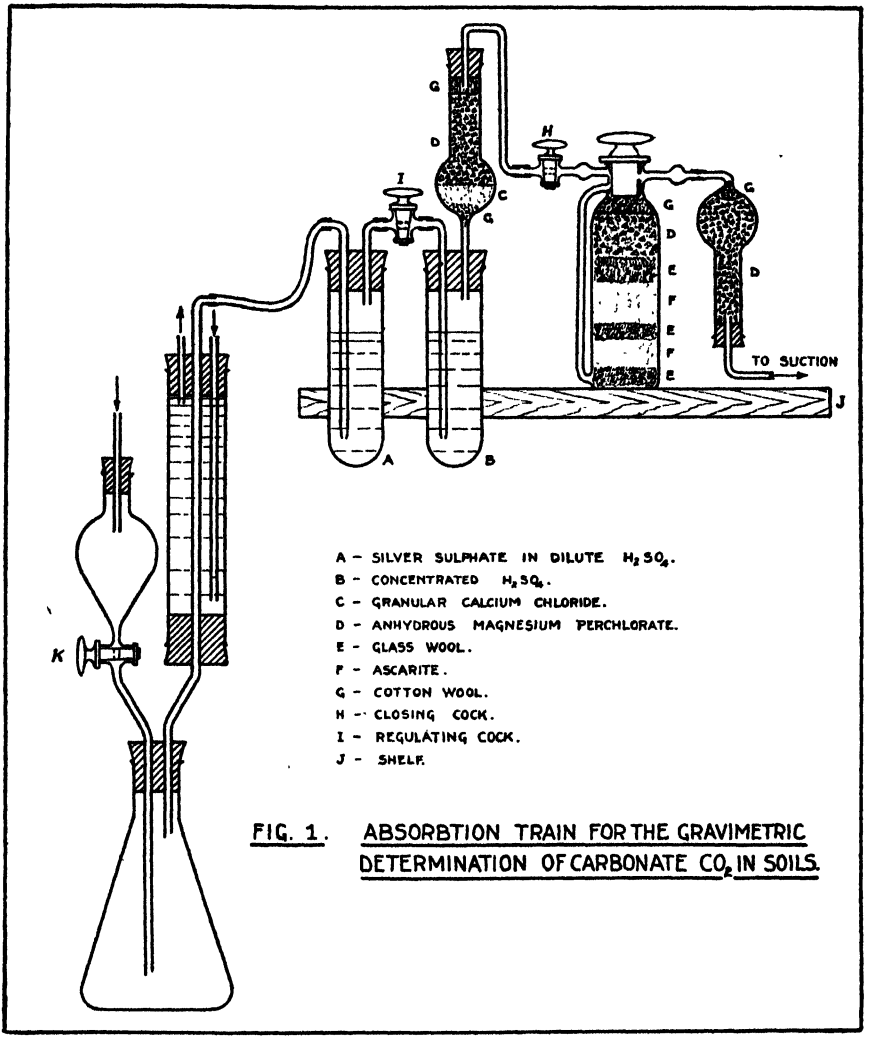


FIG. 1. ABSORBTION TRAIN FOR THE GRAVIMETRIC DETERMINATION OF CARBONATE CO_2 IN SOILS.

DETERMINATION

Grind the soil sample to pass a 60-mesh screen and shortly before analysis mix it thoroughly by rolling. Weigh from 5 to 50 gram charges, depending on carbonate content and calculate them to contain from 0.5 to 1 gram equivalence of calcium car-

[1] Willard and Smith, *J. Am. Chem. Soc.*, 44, 2255 (1922).

bonate per determination. Introduce the charge into the 300 ml. Erlenmeyer flask, close the flask tightly with the 2-hole stopper, and place on rack *A*. Introduce 75 ml. of stannous chloride-hydrochloric acid solution into the separatory funnel, and close tightly with 1-hole stopper of the air manifold *I*. Making sure the connections in the absorption train are tight, turn on the suction pump with cock *H* open and cock *I* closed, and so regulate the pump as to obtain a vacuum of 10 cm.; then open cock *I* gradually and adjust the flow of gases through the 2 mm. aperture in *A* and *B* to a rate of 5 to 6 bubbles per second. Admit the acid solution into the evolution flask by carefully opening cock *K* (Fig. 1), governing the rate of flow by the nature of the carbonate and the carbon dioxide generated. If the content of carbonate is

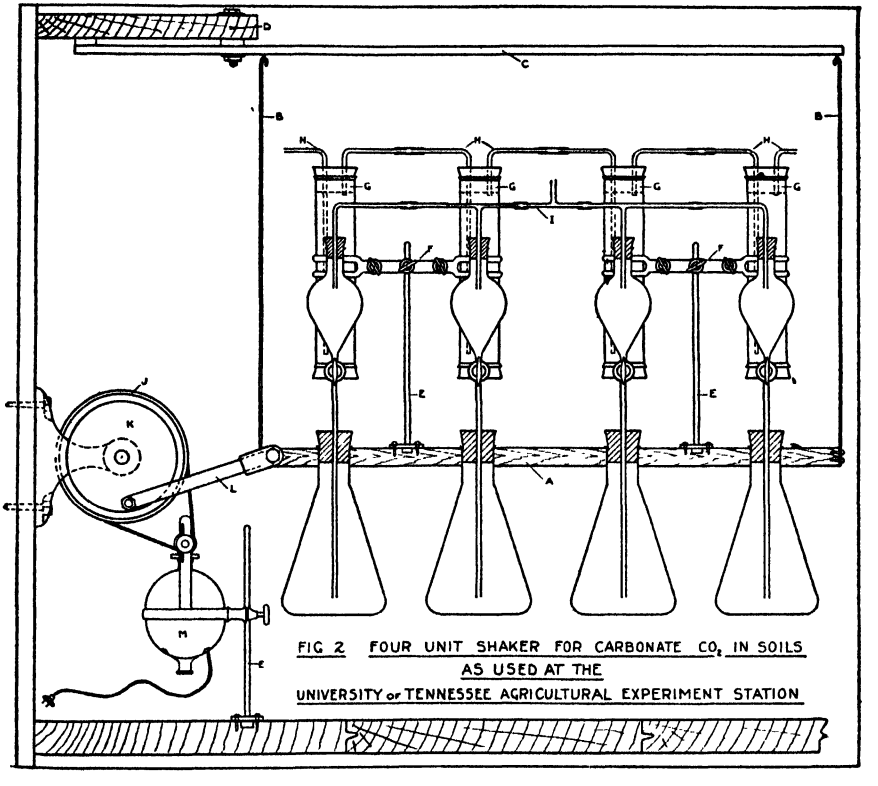


FIG 2 FOUR UNIT SHAKER FOR CARBONATE CO_2 IN SOILS AS USED AT THE UNIVERSITY OF TENNESSEE AGRICULTURAL EXPERIMENT STATION

low or resistant, admit the acid solution freely; if the evolution of carbon dioxide is too rapid, close cock *K* and start agitation gradually and intermittently. When the gas evolution has subsided, start the continuous agitation at a rate sufficient to cause the mass on the bottom of the evolution flask to be thrown back and forth during agitation. Apply heat from a Bunsen burner; see that the condenser is functioning properly; gradually increase the intensity of the heat until the liquid begins to boil; and continue boiling until gases cease to pass from the evolution flask as indicated by a dead-stop of the current in the tubes *A* and *B* of Fig. 1. Remove the flame, quickly open cock *K* to admit carbon dioxide-free air, and continue aspiration for 20 minutes to complete the determination. Close cock *H*, loosen the stopper in the evolution flask, stop suction, and remove the absorption bulb to the balance

room. At this point introduce another weighed absorption bulb to be used in the next determination, wipe with lintless cheese cloth, and allow to stand in the balance room 20 minutes. Open the bulb instantly to equalize pressure and weigh against a similar bulb serving as a counterpoise. Record the gain in weight as carbon dioxide. Run blank determinations on the reagents and the atmosphere of the flask and correct results accordingly.

SUMMARY

A study was made of the time and rate of aspiration necessary for the complete recovery of carbon dioxide in standard samples of limestones and dolomites by the official method.

The addition of stannous chloride to the hydrochloric acid made possible a rapid and complete decomposition of carbonates in soils by the application of heat without serious interference from organic matter. The procedure and apparatus are described fully.

THE NEUBAUER METHOD AS APPLIED TO THE DETERMINATION OF THE AVAILABILITY OF PHOSPHATE MATERIALS[1]

By S. F. THORNTON (State Chemist Department, Purdue University Agricultural Experiment Station, Lafayette, Ind.)

INTRODUCTION

The official neutral ammonium citrate method for the determination of phosphorus availability was originated for use with superphosphate, primarily, and for this material it has proved quite satisfactory. Recent developments in fertilizer manufacture, however, have introduced new materials for which this time-honored method is being found less applicable. At present the greatest interest is centered upon ammoniated superphosphate.

Although it has not been extensively used for this purpose, because it utilizes the solvent powers of the plants themselves, the Neubauer procedure[2] offers a method of attack which should give information of much value concerning the true availability of phosphate materials. It also offers opportunities such as are offered by no other laboratory method for studying the influence of varying conditions, such as *p*H, soil type, combination with other fertilizer materials, etc.

PROCEDURE

The soil used was a Bedford silt loam with a *p*H of 5.4, and the sand was a nutrient-free quartz sand with a neutral reaction. An amount of phosphate material equivalent to 25 mg. of total phosphoric acid in each case was mixed with the soil or sand, covered with distilled water, and allowed

[1] Fellowship supported by N. V. Potash Export My. Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1930.

[2] *Z. Pflanzenernähr. Dungung*, 8B, 219–233; Neubauer and Schneider, *ibid.*, 2A, 329–341; Neubauer, Bonewitz, and Schottmüller, *ibid.*, 12A, 108–114.

to stand for approximately two weeks. This procedure, it was thought, would make possible a maximum fixation of the phosphorus by the soil.

The Neubauer method may be given briefly as follows:

Mix 100 grams of air-dried soil with 50 grams of sand and spread uniformly over the bottom of a glass dish having a diameter of 11 cm. and a depth of 7 cm. Over this layer spread 250 grams of sand and in the center insert a glass tube to facilitate watering. In the surface of the sand layer plant 100 carefully selected rye grains that have been previously treated with a 0.25 per cent "Uspulun" solution, add 80 grams of distilled water, and maintain at a temperature of 20°C. during the vegetation period. As a blank, grow plants under similar conditions on sand alone. After 17 to 18 days wash the plants free from adhering soil and sand, dry, ash at a low temperature and determine the phosphoric acid and potash contents in the usual manner.

DISCUSSION

The accompanying table shows the phosphorus recovery from 13 phosphate materials in which both sand and soil are used. For comparative purposes, results of pot tests and of the official neutral ammonium citrate method are included. For all methods the relative percentages are based on monocalcium phosphate as 100.

In comparing recovery on sand and soil, the fixation power and the acid reaction of the soil must be given consideration.

Wolkoff,[1] in making comparative availability tests with 0.2 *N* nitric acid at intervals after completion of the reaction between soil and phosphate, found that even superphosphate became partly insoluble. The moisture content of the soil had no influence, but a rise in temperature slightly decreased the recovery of superphosphate. Ingham[2] found that subsequent solubility in 1 per cent citric acid depends more on the soil reaction than upon the previous solubility of the original materials. Recovery of superphosphate varied from 21 per cent in a very acid soil to 96 per cent in a soil containing no free acid, and it was closely related to the iron, aluminum and organic matter contents of the soils. Removal of calcium carbonate from soils high in iron and aluminum doubled the absorption, while addition of calcium carbonate to acid soils increased the availability only after 12 months. The Agricultural Experiment Station of the University of California[3] found that in certain soils having a high power for fixing phosphate, highly soluble phosphates may rapidly assume a form unavailable to plants. Ellett and Hill[4] found a similar fixation, especially by hydroxides of iron and aluminum, when the solubility was measured by means of citric acid, neutral ammonium citrate or 0.2 *N* nitric acid. As measured by plant responses, however, such fixed materials retained their availability. Likewise Conner[5] showed that phosphorus fully precipitated by iron, aluminum, and calcium may be as

[1] *Soil Sci.*, 17, 39 (1924).
[2] *S. Africa J. Sci.*, 22, 22–134 (1925).
[3] Report, 1928–29, p. 86.
[4] Am. Rpt. Va. Polytech. Inst. Agr. Exp. Sta., 1919–27, p. 22.
[5] *Am. Fertilizer*, 72, No. 8, p. 17.

Phosphorus availability as determined by the Neubauer method.

MATERIAL (25 mg. total P_2O_5)	RECOVERY				AVAILABILITY			
	SAND		SOIL		NEUBAUER METHOD		Pot Test Soil†	Ammonium Citrate
	Without Potash	With Potash*	Without Potash	With Potash*	Sand	Soil		
	per cent	*per cent*	*per cent*	*per cent*				
Mono-calcium phosphate	38.00	43.60	26.80	29.20	100	100	100	100
Di-calcium phosphate	32.40	37.20	34.00	35.60	87	124	93	96
Tri-calcium phosphate	14.00	18.00	26.00	31.60	40	103	82	25
Ammoniated oberphosphate	34.80	40.40	28.40	32.40	94	109	82	63
Ammoniated oberphosphate-residue A‡	15.60	20.40	18.00	26.00	45	79	77	18
Ammoniated oberphosphate-residue B§	11.60	18.00	15.60	16.40	37	57	68	13
Ammoniated superphosphate-residue A‡	18.00	22.00	17.20	22.00	50	70	79	27
Ammoniated superphosphate-residue B§	8.40	12.40	8.40	10.80	28	34	49	24
Rock phosphate (washed & ground)	6.00	7.60	6.00	7.60	17	24	39	4
"Disintegrated" rock phosphate	3.20	5.40	2.80	5.60	11	15		
"Precipitated" phosphate	12.40	17.20	18.00	22.80	37	73		91
"Basic" superphosphate	30.00	37.20	32.40	37.20	84	124		83
Superphosphate	32.40	35.60	33.20	34.00	85	120		95

* 25 mg. K_2O as muriate of potash.
† Results by S. D. Conner. Based on dry weight increase for barley on Bedford silt loam soil.
‡ From extraction with 100 cc. neutral ammonium citrate per 2 grams of material.
§ From extraction with 100 cc. neutral ammonium citrate per 0.5 gram of material.

available as the more soluble phosphates, when production of dry matter is used as the measure. This is especially true when the seven year average is considered.

In the present work the most significant fixation is with mono-calcium phosphate, with which there is an increase in recovery of approximately 43 per cent for sand over soil. On the other hand, with tricalcium phosphate there is an increased recovery of 80 per cent for soil as compared to sand. This increase probably is due to the much greater solubility of the tricalcium phosphate in the acid reaction of the soil. A similar tendency is exhibited by several of the other less soluble materials. Thus the high recovery on soil for di-calcium phosphate, "basic" superphosphate and ordinary superphosphate as compared to mono-calcium phosphate appears to be due to the greater fixation by the soil of the more soluble mono-calcium phosphate and to the increased solubility of the more insoluble materials produced by the acid reaction of the soil.

As an illustration of the possible effect of combination with other fertilizer materials on availability, the influence of potassium chloride additions is shown in the accompanying table. In all cases, such additions give increased values for phosphorus recovery. Although these increases are comparatively small, they are so consistent that they may be taken as indicative of a definite tendency.

SUMMARY

The availability of 13 phosphate materials in both an acid soil and as neutral sand is studied by means of an adaptation of the Neubauer method. While there is a poor correlation between such results and the results obtained by the official neutral ammonium citrate method, the correlation with the results of pot tests is such as to indicate that the Neubauer method gives very valuable information as to the true availability of different phosphates. With this method it is possible to study the influence of varying conditions, such as *p*H, soil type, combination with other fertilizer materials, etc., without consideration of which a determination of actual availability appears impossible.

POTENTIATION OF TOXICITY OF STRYCHNINE BY QUININE[1]

By RICHARD I. GRANTHAM and JAMES C. MUNCH[2] (Sharp & Dohme, Philadelphia, Pa.)

Mixtures of quinine and strychnine are frequently prescribed and large quantities of the elixirs, also of "I-Q-S," are manufactured. Although a number of chemical methods of separation and determination have been

[1] Presented at the Annual Meeting of the Association of Official Agricultural Chemists held at Washington, D. C., October, 1930.

[2] Acknowledgment is made to Justus C. Ward, Bureau of Biological Survey, Denver, Colo., for assistance in conducting the toxicity tests.

proposed from time to time, no chemical method that has come to the authors' attention has proved adequate or successful. The determination of the total alkaloids is not a particularly difficult problem, but a satisfactory chemical method for the separation of quinine from strychnine has not been developed. Under these conditions the authors attempted to develop a physiological method of assay based on the difference in pharmacological activities of the two alkaloids.

Experiments were conducted upon a large number of rats, mice, rabbits, guinea pigs and upon a smaller number of cats and dogs. Data obtained upon rats are given in the table. Further investigations are under way and will be reported subsequently.

The lethal dose of strychnine, when injected subcutaneously into white rats weighing between 100 and 400 grams, is 2.5 mg. per kilo; the lethal dose of quinine under the same conditions is 1000 mg. per kilo. Because of this great difference in toxicity it was considered that the determination of the lethal dose of mixtures of quinine and strychnine in various proportions would serve as an index of the strychnine content. In general, mixtures used in pharmaceutical preparations contain about one hundred times as much quinine as strychnine. Tests were conducted to determine the toxicity of mixtures of quinine and strychnine in various proportions ranging from 0.25:1 up to 500:1. Very unexpectedly a marked potentiation in toxicity was found. In mixtures containing 5:1 or larger ratios of quinine to strychnine, the lethal dose was found to be about 1.0 mg. of strychnine per kilo of body weight. Smaller ratios showed a minimum ethal dose of approximately 1.5 mg. per kilo of strychnine.

This potentiation of toxicity was so unexpected that the pharmacological mechanism producing such a change is being investigated further. It is interesting to note that the symptoms of strychnine poison were obtained in every instance, irrespective of the quinine-strychnine ratio. Available information would suggest an intramolecular combination of quinine and strychnine which is more toxic than either constituent alone. The development of such a complex might also explain the difficulty encountered in attempts at chemical separation.

Results to date suggest that mixtures of quinine and strychnine may not be assayed by their toxicity to rats, because of a hitherto unrecorded potentiation in toxicity.

Minimum lethal dose of mixtures of quinine and strychnine.

Subcutaneous Injections to Rats[1]

COMPOSITION OF MIXTURE RATIO IN MOLS		DOSE ADMINISTERED—MG. PER KG.														M.L.D.
Quinine	Strychnine	0.75	0.8	0.9	1.0	1.1	1.2	1 3	1.4	1.5	1.75	2.0	2.25	2.5	2.75	
0	1	—	—	—	0/1	—	—	—	—	0/1	—	0/4	2/4	5/5	1/1	2.5
1	1	—	0/1	—	0/1	—	0/1	—	1/1	—	—	—	—	—	—	1.4
5	1	—	0/2	—	0/3	—	0/2	—	2/2	1/1	—	1/1	—	—	—	1.4
10	1	—	—	—	0/5	1/3	3/3	1/1	1/1	—	—	—	—	—	—	1.2
25	1	—	—	—	0/2	1/2	2/2	—	—	—	—	—	—	—	—	1.2
50	1	2/2	0/2	2/2	2/2	—	2/2	—	—	—	—	—	—	—	—	0.9
100	1	0/1	3/3	2/3	3/4	—	—	—	—	1/1	—	1/1	—	1/1	—	0.8
150	1	—	—	0/3	0/2	2/3	2/2	1/1	2/2	2/2	1/1	—	—	—	—	1.1
200	1	—	0/2	0/2	0/2	1/1	1/1	—	—	—	—	—	—	—	—	1.1
250	1	—	0/2	0/1	2/2	1/1	—	—	—	—	—	—	—	—	—	1.0
			750		800		900		1000							
1	0		0/1		1/3		1/3		4/4							1000

[1] Numerator represents number of rats dying; denominator represents total number of rats injected.

Note on The Determination of Citric Acid[1]

In an attempt to determine citric acid in coffee by the procedure of Hartmann and Hillig for the determination of this acid in fruit products[2] it was found that alcohol-ether-soluble material was precipitated along with the pentabromacetone, giving rise to high results. The volatility of pentabromacetone at low temperature made possible the application of a procedure for removing this substance from the precipitated material by sublimation. The procedure found effective follows:

After being weighed, the Gooch crucible is replaced in the suction flask. An intake tube for aspiration with air is provided, as detailed in the method given by Hartmann[3] except that a glass coil heated in a sand bath is substituted for the sulfuric acid and soda lime containers to permit warm air to be aspirated through the crucible. The heated air is aspirated through the crucible for 10 to 15 minutes. Accurate regulation of the temperature of the sand bath is not necessary. The crucible should become distinctly warm, and it can be prevented from becoming hot by controlling the velocity of the aspirated air. The pentabromacetone removed is then determined by difference.

This procedure permits of a melting point identification of the pentabromacetone, as the volatilized compound may be conveniently collected by leading the aspirated air into a test tube surrounded by ice water suspended in a suction flask by means of a wire. A melting point can be determined by the usual means on the pentabromacetone, which has been allowed to become crystalline by standing overnight. A fairly accurate melting point may be obtained without the necessity of removing the pentabromacetone from the test tube by immersing the tube, with a thermometer attached, in a beaker of water and heating gradually, with stirring, until the crystals are seen to melt. The melting point of pentabromacetone was found to be 73°–74°C.

This procedure, tried on pure solutions containing 45 mg. of citric acid, gave 44 mg. as a mean of three determinations, as compared with 44.2 mg. as a mean of three determinations by the usual method. It permits of an accurate determination of citric acid in materials containing nonvolatile interfering substances, and a more complete identification of pentabromacetone, the specific compound formed from citric acid under the conditions of the determination.

[1] Contribution from Food Control, Food and Drug Adm., U. S. Dept. Agr by Paul A. Clifford; W.B. White, Chief.

[2] *This Journal*, 13, 99 (1930).

[3] *Ibid*, 10, 272 (1927).

EDITORIAL

AVAILABILITY OF VARIOUS FORMS OF PHOSPHATES FOR PLANT GROWTH

For many years the greater part of the phosphoric acid used in commercial fertilizers has been superphosphate prepared by acidulating phosphate rock. In this material the phosphorus is present largely as monocalcium phosphate with some dicalcium phosphate. Recent developments in the synthetic production of nitrogen compounds have made anhydrous ammonia available commercially for the production of fertilizers. This has led to the addition of free ammonia to superphosphates, resulting in the formation of less soluble forms of phosphorus.

Jacob[1] has pointed out the following economic advantages of the use of anhydrous ammonia: (1) It permits the fixation of considerable quantities of ammonia with minimum dilution of the superphosphate which is of value in the production of high analysis fertilizers; (2) it greatly improves the mechanical drilling and storage qualities of the fertilizer; (3) it reduces the rotting of fertilizer bags; and (4) it is the cheapest form of ammonia available to the fertilizer industry.

The present official method for the determination of available phosphoric acid in fertilizers has been in use without essential change for many years. It has been found to be an excellent method for superphosphates, but even the extensive work done early[2] in studying the method indicated that it was less suitable for other types of phosphorous fertilizers. The treatment of superphosphates with free ammonia reduces very markedly the amount of available phosphoric acid as determined by the present official method. This raises the question as to whether the present official method is suitable for the determination of available phosphoric acid in these ammoniated superphosphates. The object of the determination of the available phosphoric acid is to enable the evaluation of phosphoric acid for plant growth. On account of the important practical considerations involved there has recently been a renewed interest in a study of the availability of phosphate fertilizers. The Association of Official Agricultural Chemists, through its Referee on Phosphoric Acid, W. H. Ross,[3] is making extensive studies of this problem.

In an intensive study of the reactions occurring during the ammoniation of superphosphates Keenan[4] found "that the P_2O_5 precipitated by ammonia from such materials as superphosphate exists as a mixture of several calcium phosphates, largely tricalcium phosphate, but also dicalcium, and some even more basic compounds than the tricalcium phosphates are present." It has been recognized for a long time that precipitated tricalcium phosphate is much more available to plants than the finely ground rock phosphate. For a long time it was believed that the reason for the greater availability of the tricalcium phosphate was due to its physical condition. Recently Jacob[5] has pointed out that phosphate rock ($3Ca_3(PO_4)_2.CaF_2$) and tricalcium phosphate ($Ca_3(PO_4)_2$) are distinctly different chemical compounds.

Howes and Jacobs[6] have made an extensive study of the effect of size of sample, time of period of digestion and pH of the ammonium citrate solution in the determination of the available phosphoric acid in superphosphates and ammoniated superphosphates. Ross and his collaborators found that the phosphoric acid of these ammoniated phosphates was considerably more available to plants than is indicated

[1] *The Phosphorus Digest*, p 5, Nov. 1930.
[2] *Wiley's Principles and Practice of Agricultural Analysis*, Vol. II, p. 132–139 (1895).
[3] Ross and Jacob, *This Journal*, 14, 182 (1931).
[4] *Ind. Eng. Chem.*, 22, 1378 (1930).
[5] *Loc. cit.*
[6] *Ind. Eng. Chem.* Anal. Ed., 3, 70 (1931).

by the results obtained with the present official method. As a result of these studies a modification of the present official method has been proposed.

The problems arising from the development of ammoniated superphosphates stimulated interest in a study of the availability of various forms of phosphates. Parker[1] has recently presented a very good review of this work.

It is well known that the availability of a particular compound of calcium and phosphorus is dependent upon a number of factors, such as the type of soil, presence of other salts, the type of crop, etc. For instance, in certain acid soils which seem to have high fixing power for soluble forms of phosphoric acid, tricalcium phosphate may show higher availability vegetation tests than monocalcium phosphate. It is a question whether it is possible to obtain true relative values for the availability of the various forms of phosphorus which will be applicable under all conditions. The relative availability will vary with the conditions under which the fertilizer is used.

[1] Address before the Agronomy Section of the Southern Agricultural Workers, February 25, 1931.

FIRST DAY

MONDAY—AFTERNOON SESSION

Continued

REPORT ON DRUGS

By Arthur E. Paul

(U. S. Food and Drug Inspection Station, Chicago, Ill.), *Referee*

During the past year twenty-five topics were assigned to associate referees. The referee made especial attempts to close as many of these as was at all possible, particularly as the new edition of *Methods of Analysis, A. O. A. C.* is to include all methods adopted at this meeting. For this reason the referee submitted to the editing committee a number of changes and amendments to the methods which have been adopted.

The following recommendations regarding current work are made:

(1) That methods proposed under the following topics be adopted as tentative:

Radioactivity in Drugs and Water
Emodin-bearing Drugs
Mercurials
Microchemical Methods for Alkaloids
Santonin
Bioassay of Drugs
Ephedra
Menthol
Chloroform and Carbon Tetrachloride
Iodoform
Emetine
Oil of Chenopodium

(2) That study of the following topics be continued:

Crude Drugs
Emodin-bearing Drugs
Microchemical Methods for Alkaloids
Mercurials
Thymol
Bismuth Compounds in Tablets
Phenolsulfonates
Sulfonal and Trional
Guaiacol
Iodoform
Ether
Small quantities of Iodides in Mixtures
Terpin Hydrate

(3) That the following topics be considered closed and work on them discontinued, at least for the present:

Radioactivity in Drugs and Water
Santonin
Bioassay of Drugs
Ephedra
Menthol
Bromides-Chlorides
Salicylates and Other Phenols in Mixtures
Colorimetric Methods for Vitamins
Chloroform and Carbon Tetrachloride
Calcium Lactate
Emetine
Oil of Chenopodium

(4) That the following new subjects be taken up during the coming year, and that an associate referee be appointed in each case:

Belladonna Ointment and Stramonium Ointment
Bromide-Bromate Methods
Ipomea, Jalap, Podophyllum
Dextrose Solution in Ampules
Rhubarb and Rhaponticum
Calcium Gluconate
Tetrachlorethylene

The following recommendations are made by the referee in regard to the revision of *Methods of Analysis:*

(5) That on p. 379 the followed methods be inserted:

SAMPLING

Tablets and Pills

Bulk lots.—Mix the lot as thoroughly as possible without mutilating the contents; count and weigh accurately and grind thoroughly at least 100 units. Calculate the average weight per unit, and utilize the powder for analysis.

In Containers—

I. 1000 or more units.—Open and cautiously mix the entire contents without mutilation and divide into two parts, one of which is liberal for a thorough analysis; usually one-third or one-fourth is ample. Return the remainder to the container as a reserve sample. Accurately weigh and count and grind the analyst's subdivision as above.

II. 100–500 units.—If more than one container is available, weigh, count, and grind the entire contents of one of them. If only one container is available, but there is sufficient material to warrant subdividing, proceed as directed under I, otherwise weigh, count, and grind the entire contents.

III. Small containers, such as tubes of hypodermic tablets.—Choose such number of containers as will constitute a satisfactory analyst's sample, weigh, count, and grind the contents.

IV. Tablets or pills of small dosages, for example 1/100 grain of active ingredient.—The number of units necessary may be so large as to render grinding unnecessary. An entire bottleful may be required. Count the units to be employed for the determination and use them without grinding.

Soft Capsules

(Same as 36(b) of the 1925 ed.)

Ampuls

Before opening dislodge any liquid adhering in neck. Mark with a file or other suitable instrument the level of the liquid on the necks of the requisite number of ampuls, open them near the tip, transfer the bulk of the contents to a small flask, and mix. To determine the volume of contents, wash and dry the empty ampuls and fill them to the mark with water from a buret or graduated pipet.

(6) Make official the method for the determination of melting point, acetylsalicylic acid, p. 387, **19.**

Modify the official method for the determination of bromine, 388, **24(c),** to read as follows:

0.1 N bromine solution.—Dissolve 3 grams of potassium bromate and 50 grams of potassium bromide in water and dilute to 1 liter. Standardize 30 cc. of the solution, using a 500 cc. Erlenmeyer flask, following the procedure under **25,** beginning " . . . and 5 cc. of strong hydrochloric acid . . . " continuing to the last sentence, which change to read: "1 cc. of 0.1 *N* bromine solution", etc.

Make official the method for the determination of camphor, now tentative.[1]

Make official the method for the determination of arsenic in iron methylarsenates.[2]

Drop the tentative yeast method for the determination of ionic silver in silver proteinates because it is essentially the same as the U. S. P. method.

Make official Methods I and II for the determination of nitroglycerine.[3]

Make official Method 2 for the determination of cocaine[4] and print it before the other method.

Make official the method for the determination of calomel.[5]

Amend the last sentence of the method for the determination of pyramidon[6] to read: "Identify the pyramidon by means of its melting point and qualitative tests." Change the heading to "Pyramidon (Amido pyrine) Official." Then follow with the sub-heading "Quantitative Method" and the amended method. Make the method official.

Make official the microchemical tests for the following alkaloids:[7] Atropine, bromine, caffeine, cinchonidine, cinchonine, cocaine, codeine, pilocarpine, quinidine, quinine, and strychnine. Heroin, which is already official, should be printed in its alphabetical location among the above.

In addition to the foregoing, it is recommended—

[1] *This Journal*, **9**, 52 (1926).
[2] *Ibid*, **11**, 49 (1928).
[3] *Ibid.*, **10**, 47 (1927).
[4] *Ibid.*, **11**, 49 (1928).
[5] *Ibid.*, 51; **12**, 52 (1929).
[6] *Ibid*, **11**, 51 (1928).
[7] *Methods of Analysis, A.O.A.C.*, 1930.

(7) That the following comments, which constitute a part of this report, be given consideration with the respective reports of the associate referees:

CRUDE DRUGS

The associate referee is carrying on an extensive investigation of the morphology of *Aconitum* species. In connection with this study, he is devoting special attention to the formulation of methods for distinguishing between the official and allied members of this species. In accord with the associate referee's recommendation the work should be continued.

RADIOACTIVITY IN DRUGS AND WATER

The associate referee makes three recommendations, and these are approved. Attention, however, is called to his recommendation No. 2, which provides for official adoption of certain methods, omitting, it seems, the tentative stage. It is recommended that this topic be closed until further work and investigations indicate the desirability of its resumption.

EMODIN-BEARING DRUGS

The associate referee worked on two separate problems, namely, the determination of aloin and chemical methods for evaluating cascara. Before concluding this problem it was necessary to check the chemical separations physiologically. The Pharmacological Laboratory of the Food and Drug Administration consented to do this work.

It is felt that satisfactory progress has been made on the subject of aloin and the findings on cascara are of especial value.

The associate referee's recommendations for further study of aloin and the adoption of his cascara method are approved.

MERCURIALS

The associate referee's recommendations that the calomel ointment method be adopted as a tentative method and that the mercuric oxide ointment method be further studied are approved.

MICROCHEMICAL METHODS FOR ALKALOIDS

The associate referee's recommendations are approved.

TERPIN HYDRATE

The associate referee studied collaboratively the method proposed by his predecessor. The results were fair but slightly high. The method involves hand extraction, but he and one of the collaborators also used the mechanical extractor. The results were distinctly high. It is suggested by Warren that since the sample examined contained sugar the solvent probably took up a small proportion of this constituent. That would explain the slightly high results in the one case, and the still higher results in the other. The retention of water is another possibility.

Under these circumstances the combined extractions should be washed with a very small amount of water in order to remove sugar or any other water-soluble material that may have been taken up by the solvent.

The study made leaves some doubt as to whether the extractions were complete, or whether possibly the sugar extracted in the hand extractor may have compensated for slightly incomplete extraction. It is therefore recommended that this method be again studied with the above considerations in view.

SANTONIN

The method studied by the associate referee is ingenious and sound. It is regretted, however, that in submitting it to his collaborators he failed to direct that the tablets and lozenges be powdered. It is also unfortunate that of the 7 samples submitted, 5 were commercial products, the composition of which, except for label statement, was not known. However, two samples, Nos. 1 and 3, were evidently prepared by the associate referee and were, therefore, of known composition and in powder form. The results, while not entirely uniform, show that the method is capable of yielding satisfactory results when used by some of the more experienced operators.

The associate referee recommends that his method be adopted tentatively, and this recommendation is approved.

ETHER

No report was given. It is recommended that the topic be continued.

BIOASSAY OF DRUGS

The associate referee recommends the tentative adoption of methods studied by him for the preparation of a purified fluidextract of ergot and the assay of ergot. These recommendations are approved. He also recommends that the cat-eye method for mydriatics and myotics, which was made tentative in 1927, be now officially adopted. This, too, is approved.

EPHEDRA

With the approval of the recommendations made by the associate referee for this relatively new product, the association will have adopted:

An alkaloidal assay method for the crude drug.
A quantitative determination of ephedrine in tablets.
A qualitative color test for ephedrine.
A quantitative method for ephedrine in inhalants.

In addition to the recommendations made by the associate referee, it is suggested that this topic be closed, at least for the present.

THYMOL

Two years ago the associate referee devised a general method for the determination of thymol. Last year he attempted to apply this general

method to mixtures containing likely interfering substances. The work was continued this year. The associate referee also compiled procedures for thymol solutions conforming to the two National Formulary thymol preparations, *Liquor antisepticus* and *Liquor aromaticus alkalinus*, which he submitted to collaborators. Consistent results were obtained.

It is felt that the work accomplished is valuable, but that further study is necessary. It would be well to determine positively the need for having a definite alkalinity during the extraction operations. If this is essential, then directions for adjustment should be given, and probably the use of CO_2 will be found to be unnecessary. The influence of glycerin, if any, should also be studied. Then the desirable form for adoption would be an addition to the present tentative method for thymol stating the additional necessary steps in the presence of the interfering substances encountered.

MENTHOL

The associate referee's recommendation that the method described by him last year[1] be tentatively adopted is approved, and it is suggested that the topic be closed.

BROMIDES—CHLORIDES

The principal object in this study was to determine whether potentiometry would prove simple and satisfactory. The results were disappointing. It does not seem worth while to pursue this work further at this time as fairly satisfactory chemical means are already at hand.

OIL OF CHENOPODIUM

It is recommended that the Paget method, as studied during the last two years and essentially as described previously,[2] be adopted as a tentative method and that the subject be closed.

SALICYLATES AND OTHER PHENOLS IN MIXTURES

This subject was considered closed last year when a tentative method for separation was adopted. It is now recommended that it be given no further attention at this time.

SMALL QUANTITIES OF IODIDES IN MIXTURES

No report was given. Some work was done during 1929, and two methods were studied. The work was not completed however. It is therefore recommended that the topic be reassigned.

BISMUTH COMPOUNDS IN TABLETS

The associate referee studied collaboratively two methods for the determination of bismuth. The results obtained by collaborators, including those which the associate referee received after preparing his report,

[1] *This Journal*, 12, 300 (1929).
[2] *Ibid.*, 13, 336 (1930).

are not sufficiently accurate to warrant the adoption of either method. In connection with his collaborative report Warren makes some suggestions which may be of assistance. It is accordingly recommended that this subject be further studied.

COLORIMETRIC METHODS FOR VITAMINS

The associate referee and his predecessor, E. M. Bailey, made a thorough study of the available literature and information on the chemical identification of vitamins. The final conclusion is that since no methods have been evolved which would warrant study by this association, the subject be discontinued.

PHENOLSULFONATES

The interesting study reported by the associate referee shows that bromination of this product may proceed past the dibrom compound and even past the tribrom product, but that a definite final point cannot be secured as there is a tendency to break up the molecule previously. The associate referee endeavored to devise carefully controlled conditions so as to produce definite compounds, and from his results he has apparently succeeded fairly well in stopping the action with either the di- or tribrom compound.

He has devised details for the determination with the formation of the dibrom compound in view, and a limited number of determinations made by the associate referee are decidedly encouraging, but since no collaborative work was done it will be necessary to continue the study.

SULFONAL AND TRIONAL

A method for the determination of trional was proposed and studied collaboratively. No work was done on sulfonal. The method consists essentially in extracting with chloroform, evaporating, drying, and weighing. The results obtained are fairly satisfactory, but would, of course, be entirely incorrect if any chloroform-soluble excipient or other ether-soluble material were present.

It is recommended that study of this topic be continued in connection with both sulfonal and trional and that a sulfur or a melting point determination be included for identifying the chloroform extract obtained.

EMETINE

The associate referee's recommendation for the adoption of the method studied is approved.

CHLOROFORM AND CARBON TETRACHLORIDE

In 1926 Associate Referee Moraw submitted a splendid report on his pioneer work on this subject. His method was tentatively adopted. During 1928 Associate Referee Kunke slightly modified the tentative method and made provision for the determination in the presence of inorganic chlor-

ides. Briefly, the method requires chlorine determination before and after saponification, since distillation was not found to be satisfactory. More recently Rogers and Murray[1] mentioned a suggestion made by L. E. Warren that chloroform may be readily distilled from a mixture if an adequate amount of alcohol is added. The distillation then proceeds more slowly and regularly and at a lower temperature. As this simple expedient seemed sound and the authors obtained satisfactory results, it was the intention that this method be studied by this association during the present year. The results obtained on authentic samples by the associate referee are very satisfactory, but the results reported by his collaborators are very low. His further investigation shows that the low results were due to loss or decomposition of chloroform before the analysis was undertaken. The duplicate results reported by the collaborators, and particularly those from determinations made from the same bottle by two different collaborators in the Chicago Station (Glycart and Kunke) are excellent. It is felt that sufficient work has been done to show the exactness of the distillation method in the presence of chlorides to warrant its adoption.

The referee has amended the present tentative method so as to include these details.

GUAIACOL

The associate referee reviewed available literature and information bearing on this topic and investigated the more promising methods. His recommendation that the subject be continued is approved.

CALCIUM LACTATE

The U. S. P. assay, in effect, comprises titrating the alkalinity of the ash and the usual qualitative tests for lime. It is recognized that a determination of lactic acid would also be desirable. It was hoped that the present form of mechanical extractor might solve the problem, but the associate referee's results are negative.

It is recommended that this topic be considered closed until more promising work is recorded.

IODOFORM

The associate referee recommends that the method devised for the determination of iodoform be adopted as tentative and applied to the product in mixtures, especially ointments. The referee approves.

REPORT ON CRUDE DRUGS

By H. W. YOUNGKEN[2] (Massachusetts College of Pharmacy, Boston, Mass.), *Associate Referee*

The problem undertaken this year was that of formulating concise directions for distinguishing the roots of the official from allied species of

[1] *Am. J. Pharm.*, 101, 654 (1929).
[2] Presented by J. F. Clevenger.

Aconitum, especially those offered for entry into this country as official.

The associate referee, realizing the need of unmistakably authentic materials as a basis for this study, made considerable effort to obtain a supply from the U. S. Department of Agriculture and from various drug gardens in this country but with little success. Later he communicated with several gardens aboard, but he has not received the materials requested.

He is now studying the roots of two varieties of *A. napellus* together with *A. fischeri* and *A. lycoctonum* which he collected during the late summer of this year from a nursery in Northern New Jersey and expects to study materials from European and Indian gardens as soon as it is received.

It is accordingly recommended[1] that the problem of aconite root be continued.

REPORT ON RADIOACTIVITY IN DRUGS AND WATER

By J. W. SALE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

A brief summary of the A. O. A. C. work on radioactivity may be of interest. In 1924 detailed methods for the qualitative determination of radioactivity of samples in solid form and for the quantitative determination of radioactivity of clear solutions were described[2] by the associate referee and adopted[3] by the association as tentative methods. In 1926 the preparation for quantitative analysis of three kinds of samples completely soluble in acids and of four kinds of samples insoluble or incompletely soluble in acids was described.[4] In 1927 the composition of several kinds of samples to be submitted later to collaborators was described.[5] In 1929 the results of collaborative work on the water samples were reported.[6] These results were regarded as satisfactory. Additional results obtained by collaborators will be reported here. The slow progress made on the collaborative work has been due to the inability of the associate referee to find experienced analysts to examine the samples. Only a few laboratories are equipped to do this type of work.

The samples of salt and kaolin which were subjected to collaborative testing this year were those described in the associate referee's report of 1927.[4] They were prepared for analysis by the procedures described in the report of 1926[3] under the heading "Preparation of Sample": *A* (a) and *C* (salt sample), *B* (b) (1) and (2), and *C* (kaolin sample). They were

[1] For report of Subcommittee B and action of the association, see *This Journal*, **14**, 50 (1931).
[2] *This Journal*, **8**, 531 (1925).
[3] *Ibid.*, 267.
[4] *Ibid.*, **10**, 362 (1927).
[5] *Ibid.*, **11**, 342 (1928).
[6] *Ibid.*, **13**, 308 (1930).

analyzed by the methods described in the report of 1924.[1] The results obtained are given in Table 1.

TABLE 1:

*Radium in salt and kaolin samples.**

	SALT SAMPLE		KAOLIN SAMPLE	
Collaborator	*Found*	*Difference*	*Found*	*Difference*
R. G. Fulton	3.13	+0.07	3.97	−0.11
	3.16	+0.10	4.11	+0.03
	3.01	−0.05	3.95	−0.13
C. H. Badger	2.91	−0.15	3.97	−0.11
	2.87	−0.19		
Max.	3.16		4.11	
Min.	2.87		3.95	
Average	3.00		4.00	
Present	3.06		4.08	

* Results are expressed in millimicrograms per 5 grams of salt and per 0.25 gram of kaolin.

The data given in Table 1, together with the results on the water sample reported in Table 1 of last year's report, show that the proposed methods for the preparation and analysis of radioactive water, salt and kaolin samples give satisfactory results. The character of these three samples is such that the methods can be regarded as generally applicable to most samples that the analyst is likely to encounter.

The associate referee was fortunate in interesting Professor Schlundt of the University of Missouri in testing the proposed methods, and Ralph G. Fulton, a graduate student working under the direction of Professor Schlundt, not only analyzed the collaborative samples according to the methods submitted by the referee but he conducted a complete independent check of these methods by comparing them with two other methods for determining radium as radon. He also applied the methods to a refractory cyrtolite ore which was probably more difficult to analyze than the kaolin sample submitted. Schlundt and Fulton concluded as a result of their work that the methods sent to them by the associate referee are reliable. A report of this work with this conclusion appeared in the November issue of *This Journal*.[2]

In view of the work cited the associate referee is of the opinion that the association should adopt as official the methods for the preparation and analysis of radioactive samples given in the reports of 1924 and 1926.

In the report of 1924 and in later reports it was recommended that the preparation of a standard stock solution of radium be described. It appears that it will not be necessary to do this as salts and solutions of known radium content can now be purchased on the market, and if the analyst will calibrate his electroscopes with at least two such solutions

[1] *This Journal*, 8, 531 (1925).
[2] 13, 497 (1930).

or salts originating from two independent sources, there can be little doubt of the accuracy of the calibration if the results are found to agree.

RECOMMENDATIONS[1]

It is recommended—

(1) That the following changes be made in the methods referred to in Recommendation 3 and published in Vol. VIII of *This Journal:*

Page 531.—Under Reagent (c) delete the statement, "a description of a method of preparing the standard stock solution of radium is in course of preparation. Change "100 cc." (5th line) to "200 cc." Change "80 cc." (7th line) to "160 cc." Other editorial changes were made for the sake of clarity.

(2) That the methods for the preparation for analysis of radioactive samples described in the report of 1926 be adopted as official (first action).

(3) That the tentative methods for the calibration of electroscopes and for the analysis of radioactive samples described in the report of 1924 be adopted as official (first action) after being revised as indicated in Recommendation 1. These methods were adopted as tentative in 1924.

EMODIN-BEARING DRUGS

By ELGAR O. EATON[2] (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

CASCARA

No chemical collaborative work was done last year. However, the Pharmacological Laboratory, in conjunction with the Drug Research Unit, did work in research with a view to showing whether or not the method[3] proposed in fact evaluated the drug. Their report shows that the method is of value and in fact is a good index of the drug's activity. More collaborative work on the chemical methods is underway.

ALOIN

The following method for the determination of aloin was submitted to collaborators:

(This procedure is applicable to mixtures containing cascara, rhubarb, senna and other acid hydrolyzable anthraglucosides, as well as resins and phenolphthalein with aloin or aloes.)

Take sufficient of the powdered material, dried for 1 hour at 110°C. (or of a dealcoholized solution of a liquid) to insure approximately 0.5 gram of aloin (or 1.0 gram of aloes). Dissolve in water with the aid of a few cc of 5 per cent sodium hydroxide solution. Transfer to a 100 cc volumetric flask, dilute to about 75 cc. and make acid at once with hydrochloric acid (as aloin is attacked by alkali). Dilute to mark and add a few glass beads if much undissolved material is present. Shake to insure solution of aloin. Filter, and transfer a 40 cc. aliquot to which is added 5 cc.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 50, 85 (1931).
[2] Presented by C. K. Glycart.
[3] *This Journal*, 13, 310 (1930).

of concentrated hydrochloric acid to a continuous extraction apparatus (Type C, No. 1,[1] which is previously charged with chloroform. Reflux to exhaustion (about 2 hours). Disconnect the apparatus and transfer all the aqueous solution to a separatory funnel, discarding the chloroform. Saturate the solution with salt and shake out five times with 30 cc. portions of chloroform-alcohol mixture (2+1).

Combine the solvent and wash with 1 cc. of water to which is also added 1.0 gram of calcium carbonate, or more if necessary, to insure an excess (as shown by undissolved material). Filter, evaporate, add 5 cc. of chloroform, evaporate, dry at 110°C. for 1 hour, cool, and weigh. Weight = aloin in aliquot taken.

As a check, acetylize the aloin by dissolving in acetic anhydride (about 10 cc.) and adding an excess (about 2 grams) of powdered sodium acetate anhydrous (see U.S.P. X, p. 482), and boil for 5 minutes over an electric hot plate in a hood. Evaporate in a hood with a good draft to apparent dryness. Add 10 cc. of water and heat several minutes. Transfer with the aid of 20 cc. of chloroform to a separatory funnel and shake out with two additional 10 cc. portions of chloroform (the aloin hexylacetate formed is soluble in chloroform). Combine chloroform and filter. Evaporate, add 10 cc of chloroform, evaporate, dry at 110°C. for 1 hour, cool, and weigh. Weight $\times 0.615$[2] = aloin.

The results were encouraging, but they showed the method needed refinements. These were made, and further samples will be sent to collaborators.[3]

REPORT ON MERCURIALS

By ROBERT S. ROE (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The work on mercurials this year consisted in the study of methods for calomel ointment and mercuric oxide ointment.

CALOMEL OINTMENT

The following method, which was submitted to collaborative study, is merely an adaptation of the tentative A.O.A.C. method for calomel tablets, which is similar in principle to the U.S.P. method for the determination of calomel.

Weigh accurately about 1 gram of the ointment and transfer to a 250 cc. glass-stoppered Erlenmeyer flask. Treat with about 50 cc of chloroform. When the base is dissolved, decant through a dry, closely packed asbestos mat in a Caldwell crucible, using light suction. Wash the flask and contents several times with 20 or 30 cc. portions of chloroform, decanting through the crucible.

Allow any residual chloroform in the flask to evaporate and transfer the asbestos mat and contents to the flask. (Wipe the sides of the crucible and the mouth of the flask with a damp piece of filter paper and add to contents of flask.)

To the contents of the flask add 2.5 grams of potassium iodide and 50 cc of 0.1 *N* iodine solution. Stopper and mix well. Let stand about 1½ hours or until solution of the calomel is complete, with frequent and fairly vigorous agitation.

Titrate with 0.1 *N* sodium thiosulfate, adding 1 or 2 cc. in excess and using starch indicator. When all traces of iodine have disappeared, titrate back with the standard

[1] *Ind. Eng. Chem.*, 17, 612 (1925).
[2] *J. Chem. Soc.*, 52, 553 (1930).
[3] For report of Subcommittee B and action of the association, see *This Journal*, 14, 50 (1931).

iodine solution until a blue color is obtained. 1 cc. of 0.1 *N* iodine represents 0.02361 grams of calomel. Report as percentage of calomel.

PREPARATION OF SAMPLE

The sample used for collaborative study was prepared by thoroughly triturating in a mortar 60 grams of calomel with 140 grams of white petrolatum. The calomel used was a commercial sample of U.S.P. grade.

The results obtained by the collaborators are given in Table I.

TABLE 1.

COLLABORATOR	CALOMEL *per cent*
F. L. Hart	29.36
	(29.05)*
	29.36
	(29.20)
	30.56†
	(30.56)†
	30.18†
	(29.84)†
	29.84†
	(30.00)†
W. F. Kunke	30.05
	(30.14)†
A. W. Hanson	30.75
	30.13
	30.94
F. C. Sinton	29.91
	29.91
	30.01

* No excess thiosulfate was used to obtain all the results given in parentheses.
† Two hours' oxidation.

When the amount and strength of iodine solution and the time of oxidation were varied, the following results were obtained:

REMARKS BY COLLABORATORS

F. L. Hart.—It is believed that the period of standing should be 2 hours instead of 1½ hours. Use of 0.2 *N* iodine does not seem to increase the speed of the reaction.

W. F. Kunke.—I am satisfied that 20 cc. of 0.1 *N* iodine is sufficient and 15 minutes reaction period is long enough. Titrations made without adding excess 0.1 *N* $Na_2S_2O_3$ apparently give just as good results as when excess is added and titrated back with iodine; *consequently the small piece of filter paper* and *the asbestos* do not appear to seriously interfere with the first end point.

F. C. Sinton.—The method was followed; 0.1 *N* iodine was used, and the standing period was about 1½ hours. No difficulty was encountered, and the method seems satisfactory.

DISCUSSION

The results reported seem to be satisfactory and within the accuracy desired for this product. It will be noted, however, that the collaborators are not agreed as to the time necessary for the completion of the oxidation. As submitted, the method calls for a treatment with iodine solution for "about 1½ hours or *until solution of the calomel is complete* with frequent and fairly vigorous agitation." The use of this method in assaying various calomel and mercurous iodide preparations has indicated that in general 1½ hours' oxidation is about right, although greater or less time may be required, depending perhaps on the amount of agitation, the temperature of the solution and possibly also the degree of fineness of the calomel. The end point of the reaction is indicated by the disappearance of particles of calomel which are fairly readily observable on the bottom of the flask. The time was given more as a guide than as a definite requirement, as it is recognized that varying conditions may alter the period required.

As to the volume of 0.1 *N* iodine necessary, 30 cc. would seem to be sufficient rather than the 50 cc. called for, judging from the results given in Table 2. This would conform to the amount used in this method as applied to calomel and mercurous iodide tablets.

TABLE 2.

Analyst	0.2 *N* I		20 cc. of 0.1 *N* I		30 cc. of 0.1 *N* I
	1½ hrs.	2 hrs.	1½ hrs.	15–20 min.	1½ hrs.
F. L. Hart	29.56 (29.75)* 29.36 (29.41)*	30.02 (30.44)*			
W. F. Kunke			30.14 (30.02)*	30.43 (30.34)* 30.40 (30.34)* 30.64* (30.57)*	
R. S. Roe					29.68 30.31 29.57 30.10

* No excess thiosulfate used.

RECOMMENDATION[1]

It is recommended that 30 cc. of 0.1 *N* iodine be specified rather than 50 cc. and that the method be adopted as tentative for the assay of calomel ointment.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 50 (1931).

MERCURIC OXIDE OINTMENT

The method submitted for collaborative study, a modification of the U.S.P. methods for mercuric oxide and mercurial ointments, is the following:

DETERMINATION

Weight accurately about 10 grams of the ointment. Transfer to a 300 cc. Erlenmeyer flask. Add 20 cc of water and 20 cc. of nitric acid. Heat gently almost to boiling. Agitate, and when the evolution of brown fumes ceases, add about 100 cc. of water and 2 or 3 cc. of ferric ammonium sulfate T.S. indicator. Titrate with 0.1 *N* ammonium or potassium thiocyanate. Again warm to melt the base, shake well, and add additional standard thiocyanate if necessary to restore the red color. 1 cc. of 0.1 *N* NH_4SCN represents 0.01083 gram of mercuric oxide. Report as percentage of mercuric oxide.

REAGENTS

(a) *Thiocyanate solution.*—0.1 *N*. Dissolve 11 grams of potassium thiocyanate (or 10 grams of ammonium thiocyanate) in 1000 cc. of distilled water. Standardize with 0.1 *N* silver nitrate.

(b) *Ferric ammonium sulfate T.S.*—Dissolve 8 grams of ferric ammonium sulfate in sufficient distilled water to make 100 cc.

PREPARATION OF SAMPLE

The sample was prepared by thoroughly mixing the following ingredients in a mortar: Yellow mercuric oxide U.S.P., 5 grams; hydrous wool fat, 50 grams; petrolatum, 445 grams.

TABLE 3.

COLLABORATOR	HART	KUNKE*	HANSON	ROE	SINTON*	FREEMAN*
Percentage of HgO	1.04 1.02 1.00	1.27 0.98 1.12 1.27	0.98 0.99	0.97 0.95 0.95		

* Report method unsatisfactory.

COMMENTS OF COLLABORATORS

W. F. Kunke.—Four 10 gram samples were taken, and titrations were made with 0.1 *N* NH_4CNS after successive heatings.

SAMPLE NO.	1st	2nd	3rd	4th	5th	6th	TOTAL 0.1 *N* NH_4CNS	FOUND HgO
	cc.	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*cc.*	*per cent*
1	8.4	0.4	1.0	0.45	0.55	0.4 0.25 0.1 (cold)	11.75	1.27
2	8.28	0.3	0.2	0.1	0.1	—	9.0	0.975
3	7.0	0.65	0.3	0.6	0.6	0.5+ 0.5	10.5	1.12
4	7.9	1.65	0.55	0.5	0.5	0.45+ 0.35	11.7	1.27

In view of the results obtained, I carried out several experiments which might throw light on the trouble.

First.—0.1083 gram of HgO required 10.00 cc. of NH_4CNS equivalent to 100 per cent of HgO. This was done to see if I was acquainted with the end point.

Second.—5 grams of petrolatum, 5 grams of wool fat, and filter were treated according to the proposed method (just as though HgO were present). This blank required 0.25 cc., 0.2 cc., 0.1 cc., 0.1 cc., 0.1 cc., and 0.1 cc. of 0.1 *N* NH_4CNS after successive heatings, or a total of 0.85 cc.

Third.—0.1083 gram of HgO (yellow), 1 gram of wool fat, 9 grams of petrolatum. Run as specified by proposed A.O.A.C. method and required 9.8 cc., 0.4 cc., 0.15 cc., and 0.15 cc. of 0.1 *N* NH_4CNS after successive heatings, respectively, or total of 10.50 cc. 0.1 *N* NH_4CNS equivalent to 105 per cent of HgO found (on basis of 0.1083 gram used).

The last experiment simulates yellow mercuric oxide ointment.

F. L. Hart.—Method appears satisfactory. The fatty material dissolved in nitric acid somewhat obscures the end point, but with care concordant results may be obtained.

N. E. Freeman.—With the method you submitted and 10 grams of sample I used about 11 cc. of 0.1 *N* thiocyanate for the first end point, but as soon as I heated the solution to remelt the base, I added in one instance over 110 cc. of thiocyanate and in another instance nearly 100 cc. without obtaining a permanent red color. I believe the nitric acid present must have been oxidizing my thiocyanate as fast as I added it as the fumes and odor of nitric oxide were in evidence after considerable thiocyanate had been added.

DISCUSSION

From the results and comments it is evident that the method is not satisfactory. It is apparent from the work reported by Kunke that the base used absorbs or reacts to a greater or less extent with the thiocyanate, nullifying the end point. Apparently when the operation is carried out rather rapidly good results are secured. In its present form the method is not suitable.

RECOMMENDATION[1]

It is recommended that further study be made of methods for the assay of mercuric oxide ointment.

REPORT ON MICROCHEMICAL METHODS FOR ALKALOIDS

By C. K. GLYCART (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The work on microchemical methods for the identification of alkaloids included further collaborative study on atropine, pilocarpine and ephedrine, and also a preliminary study of aconitine, arecoline, physostigmine and yohimbine.

In the 1928 report[2] on microchemical methods, some of the collabora-

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 50 (1931).
[2] *This Journal*, 11, 353 (1928).

tors stated that the tests for atropine and pilocarpine were inconclusive. The tests were repeated by the associate referee, and the methods originally described were adopted as tentative[1] in 1929. To show whether these tests were sufficiently reliable to warrant recommendation for final adoption, the alkaloids atropine and pilocarpine were subjected to collaborative study for the second time.

The directions for the tests and control specimens, consisting of atropine sulfate, pilocarpine, hydrochloride and ephedrine hydrochloride, also samples labeled Nos. 1, 2, 3 for identification were sent to the collaborators.

No. 1 contained a 1:100 ephedrine solution, No. 2 a 1:100 atropine solution, and No. 3 a 1:100 pilocarpine solution.

The bismuth and potassium iodide reagent for the identification of ephedrine was prepared with bismuth subnitrate in place of bismuth nitrate as directed for Kraut's reagent.

MICROCHEMICAL TESTS FOR ALKALOIDS

REAGENTS

(a) *Bismuth and potassium iodide solution.*—Dissolve 2 grams of bismuth subnitrate in 10 cc. of nitric acid (1+1). Dissolve 7 grams of potassium iodide in a little water. Mix the solutions and dilute with water to 50 cc.

(b) *Platinic chloride solution.*—5 per cent.

(c) *Wagner's reagent.*—Dissolve 1 gram of iodine and 5 grams of potassium iodide in a few drops of water, then dilute to 100 cc.

(d) *Millon's reagent.*—Dissolve metallic mercury in an equal weight of strong nitric acid and dilute with an equal volume of water.

PREPARATION OF SAMPLES

(a) *Controls.*—Dissolve 1 mg. of the pure alkaloidal salt in two drops of water to make an approximately 1–100 solution.

(b) *Alkaloids in compounds.*—Separate the alkaloid in pure form by extracting from ammoniacal solution with a suitable immiscible solvent, and evaporate the solvent. To 1 mg. of the residue add, drop by drop, 0.1 *N* hydrochloric acid, avoiding an excess of acid, and dilute with water, if necessary, to approximately the same alkaloidal concentration as used in (a).

(c) *Hypodermic tablets.*—Dissolve a portion of a tablet in water and dilute with water to approximately the same alkaloidal concentration as used in (a).

Characteristic microchemical test for alkaloids

ALKALOID	REAGENT	DESCRIPTION OF CRYSTALS
Ephedrine	Bismuth and Potassium Iodide	Long, brown, radiating and interlacing needles
Atropine	Wagner's	Small, dark, angular plates in great numbers
Pilocarpine	Platinic Chloride	Thin, yellow, irregular overlapping plates

[1] *This Journal*, 12, 284 (1929).

IDENTIFICATION

Place a drop of the alkaloidal solution on a clean glass slide; add a drop of reagent by means of a clean glass rod; and, without stirring or covering, examine under the microscope, using low power. A magnification of 100–150 is suitable. Note the kind of crystals formed, and compare their characteristics with the descriptions given and then with a control.

RESULTS AND COMMENTS

F. C. Sinton, Food and Drug Administration, Chicago, Ill.—No. 1, *Ephedrine;* No. 2, *Atropine;* No. 3, *Pilocarpine.*

When a control was used no difficulty was experienced in identifying the alkaloids. It was noted that in the case of ephedrine with the bismuth and potassium iodide reagent, in addition to the long radiating needles, groups of brown-red plates mostly triangular were formed. Pilocarpine seems to require some standing before crystallization occurs, at least with the concentration of the unknown.

Newell E. Freeman, Food and Drug Administration, New Orleans, La.—No. 1, *Ephedrine.* No results to report on the bismuth reagent as the necessary ingredients were not readily available. *Millon's reagent:* The precipitate in this instance was so dense that it appeared amorphous. However, a few rather large crystals did form. They presented the appearance of irregular bundles of short rods. No. 2, *Atropine. Wagner's reagent and hydrochloric acid:* The crystals I obtained would perhaps be better described as innumerable small needles with a tendency to form rosettes. No. 3, *Pilocarpine. Platinic Chloride:* These crystals corresponded to the description given, and no difficulty should be encountered in recognizing them even without comparison with the control.

E. O. Eaton, San Francisco, Calif.—No. 1, *Ephedrine,* present; No. 2, *Atropine,* indicated; No. 3, *Pilocarpine,* present. The crystals are fairly well described, except for atropine. Microphotographs would be of great value.

Charles C. Fulton, U. S. Treasury Department, Omaha, Neb.—No. 1, *Ephedrine.* (a) *Bismuth and potassium iodide.*—I observed two kinds of crystals. Part of the precipitate usually crystallizes first in small orange plates, but the long brownish needles soon form and predominate, except perhaps in the most dilute solution that will give a precipitate. In making the reagent the bismuth iodide did not at first dissolve—possibly I did not weigh the ingredients accurately, but the amount of potassium iodide given in the formula seems rather small. I added a little more KI and secured a satisfactory reagent. In my opinion the test affords satisfactory identification of ephedrine, and it is probably about as good an alkaloidal precipitation test as can ever be found for this alkaloid. (b) *Millon's reagent.*—I could obtain no results whatever with this reagent. The only crystals obtained appeared to be due to the reagent thrown out of solution by dilution.

No. 2, *Atropine. Wagner's reagent.*—This form of the iodine reagent is probably not the best reagent for atropine, but it will give characteristic crystals by which atropine can be identified. The solution submitted was too concentrated for good results; an atropine solution as strong as 1:100 gives variable results, depending on the relative size of the drops of alkaloidal solution and reagent. The reagent is very sensitive to atropine. From fairly dilute solutions it gives great numbers of very small crystals, plates or grains. These appear dark, even black, under low power, but under high power one can see that many, if not most, are light yellow. I think that these crystals can be recognized by anyone who has seen them a time or two and knows what to expect from atropine; but I would recommend the use of the high power lens for their examination.

No. 3, *Pilocarpine. Platinic chloride.*—The solution submitted gave no immedi-

ate precipitate, but on allowing it to stand for some little time the characteristic crystals appeared. The great defect of the test is its lack of sensitiveness. The crystals can be easily recognized if obtained.

W. F. Kunke, Food and Drug Administration, Chicago, Ill.—No. 1, *Ephedrine.* (a) *Bismuth and potassium iodide:* Long, brown radiating or branching needles which also interlace. (b) *Millon's:* Round clusters of very small needles resembling a thistle burr.

No. 2, *Atropine. Wagner's:* Small, dark brown, comparatively long angular plates in large number.

No. 3, *Pilocarpine. Platinic Chloride:* Thin, pale yellow, very irregular plates which frequently overlap and present jagged edges.

These tests are characteristic and are a ready means of identifying the alkaloid concerned.

DISCUSSION

The findings on atropine and pilocarpine this year show that the tests, as directed, are adequate for their identification. The crystals formed by the bismuth and potassium iodide reagent with ephedrine are characteristic, and the tests are entirely satisfactory.

RECOMMENDATIONS[1]

It is recommended—

(1) That the microchemical tests for atropine and pilocarpine, now tentative, be adopted as official (first action).

(2) That the microchemical test for ephedrine be made tentative with a view to adoption as official.

(3) That the alkaloids aconitine, arecoline, physostigmine and yohimbine be studied collaboratively next year.

REPORT ON TERPIN HYDRATE

By C. B. STONE (U. S. Food and Drug Administration, Minneapolis, Minn.), *Associate Referee*

Collaborative work was done on the method reported by the associate referee[2] in order to make it official if the results warranted.

The elixir used was prepared according to the procedure outlined in the National Formulary,[3] 17.5 grams of terpin hydrate per liter being used. The method reported by Harrison was used without any change.[4]

The results obtained are given in the following table:

The results secured by the collaborators are fair; on the average they are about 1.2 per cent too high. One criticism, which has already been referred to by Harrison, is that it is a determination of chloroform-soluble extractives.

The method has now been tried out for two years and therefore can be

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 51 (1931).
[2] *This Journal*, 11, 358 (1928).
[3] 5th ed., 1926, p. 48.
[4] *Ind. Eng. Chem.*, 17, 612 (1925).

Collaborator	*grams per 100 cc.*		
Wm. F. Kunke	1.798		
Chicago, Ill.	1.783		
	1.775		
	1.765	Av.	1.780
M. J. Gnagy	1.750		
Minneapolis, Minn.	1.753		
	1.761	Av.	1.755
Guy C. Frary	1.758		
Vermilion, S.D.	1.807	Av.	1.782
Mr. Dalbom	1.731		1.731
Vermilion, S.D.			
Robert L. Herd	1.786		
Baltimore, Md.	1.771	Av.	1.779
C. B. Stone	1.767		
	1.762		
	1.776	Av.	1.768
Average			1.771

considered useful for the determination of terpin hydrate in the elixir and in simple mixtures where terpin hydrate is present.

The Palkin-Watkins liquid extractor[1] was tried out by Kunke and the referee with only fair results. Chloroform was used as the solvent by both collaborators. The results follow.

	grams per 100 cc.		
Wm. F. Kunke	1.885		
	1.825	Av.	1.855
C. B. Stone	1.895		1.895

The results secured were considerably higher than those obtained with the proposed method when the same solvent was used. The mechanical extractor seems to dissolve more of the chloroform-soluble substances than the hand extraction method.

The referee tried carbon tetrachloride as a solvent, using the Palkin-Watkins extractor. The results were very low, and the crystal formation was very different from that expected of terpin hydrate. It is possible that some change had taken place during the extraction process with the carbon tetrachloride as a solvent.

In view of the results secured the associate referee wishes to recommend:[2]

[1] *Ind. Eng. Chem.*, 17, 612 (1925).
[2] For report of Subcommittee B and action of the association, see *This Journal*, 14, 51 (1931).

(1) That the proposed method be adopted as a tentative method.

(2) That in view of the results secured this particular problem be considered closed.

REPORT ON SANTONIN

By HENRY M. BURLAGE (School of Pharmacy, Purdue University, Lafayette, Ind.), *Associate Referee*

The 1928 report[1] shows that the existing gravimetric methods are very unsatisfactory. After extensive study two modifications[2] of the Kariyone and Kimura method[3] and Langer's method[4] were proposed. The results obtained show that the modified Kariyone and Kimura method was satisfactory for non-fatty mixtures if a correction was applied. This correction represents the alkali required by ingredients other than santonin in the sample. For this reason, the method is limited to samples which are composed of known amounts of santonin as well as other ingredients. It was recommended that this method be adopted for the assay of non-fatty mixtures containing santonin, whose exact composition are known. However, Langer's method (modified) showed greater promise, and it was recommended that this method be adopted for the assay of santonin in non-fatty mixtures and be adopted tentatively for the assay of santonin in fatty mixtures. The comments on these results are given in the previous report. The action taken by the association[5] was that the work be continued.

Because Langer's method (modified) showed greater possibilities than the Kariyone and Kimura method (modified), it was decided to study the Langer method collaboratively.

The collaborative work was limited to the following persons who had assisted in previous studies: C. O. Ewing, L. E. Warren, Liberino Patricelli, and the associate referee.

PREPARATION OF SAMPLES

Samples in the form of a fine powder were prepared as directed previously.[6] Sample 1 was a non-fatty mixture containing 2.94 per cent of santonin. In each case 5.0000 grams was weighed out. Sample 3 was a fatty mixture containing 3 per cent of santonin, and in each case 1.5000 grams was taken. Sample 5 consisted of 5 lozenges, each lozenge containing ½ grain (0.0325 gram) of santonin and ½ grain of calomel. Sample 7 consisted of tablet triturates containing 1/10 grain (0.0065 gram) of santonin and 1/10 grain of calomel; 2 tablets were used as a sample. Sample 9 was composed of tablet triturates containing 1 grain (0.065 gram)

[1] *This Journal*, **12**, 284 (1929).
[2] *Ibid.*, **13**, 318 (1930).
[3] *J. Pharm. Soc. Japan*, No. 405, 927, *Pharm Weekbl.*, **58**, 1299 (1921), Yearbook Am. Pharm. Assoc., **10**, 274 (1921).
[4] *Apoth. Ztg.*, **43**, 815, (1928); *C.A.*, **22**, 3488 (1928).
[5] *This Journal*, **13**, 65 (1930).
[6] *This Journal*, **12**, 284 (1929).

TABLE 1.

Percentages of santonin by Langer's method (modified).

NO. OF SAMPLE	PERCENTAGE OF SANTONIN IN ENTIRE SAMPLE (GRAVIMETRIC)				PERCENTAGE OF SANTONIN IN ENTIRE SAMPLE (VOLUMETRIC)	
	B	E*	W†	P	B	E
(1)	2.93	2.94+	2.93 (3.03)	2.96	2.80	2.91+
	2.94	2.95−	2.93 (3.43)		2.74	2.92+
	2.98	2.98+			3.11	2.90+
Av.	2.95	2.98−	2.93		2.88	2.92
Grand Av.			2.95+		2.90	
(3)	2.59	3.00	2.80 (3.04)	2.70	2.49	2.80+
	2.70	3.00+	2.54 (3.43)	2.83	2.34	3.05−
	2.53	3.00+	2.69 (3.24)		3.52	2.56
	2.69				3.23	
	3.03					
Av.	2. 1	3.00	2.68	2.77−	2.90−	2.81−
Grand Av.			2.87		2.85	
(5)	3.03		3.06 (3.18)			
	3.00		2.98 (3.21)			
Av.	3.01+		3.02			
Grand Av.			3.01+			
(7)	7.03		6.85 (7.10)	6.86	7.03	
	7.07		6.90	6.17	6.97	
Av.	7.05		6.88−	6.52−	7.00	
Grand Av.			6.82−			
(9)	40.28		38.80 (48.40)	44.17	41.68	
	40.81		38.30 (44.10)	42.81	40.05	
	39.90				42.49	
Av.	40.33		38.55	43.49	41.41−	
Grand Av.			40.80−		41.41−	
(11)	17.81		17.36 (17.47)	16.52	17.86	
	17.56		16.83 (17.18)	17.12	16.67	
	17.55		17.11 (17.28)		16.57	
Av.	17.64		17.35	16.82	17.03+	
Grand Av.			17.27		17.03+	
(13)	80.40		80.57 (82.04)	79.29	82.48	
	81.98		81.23 (82.30)	84.53	87.20	
	82.43		81.64 (81.69)		87.29	
Av.	81.60		81.15−	81.91	85.66−	
Grand Av.			81.55		85.66−	

* Calculated.
† See collaborators' comments.

of santonin and 1 grain of calomel; 2 tablets were used as a sample. Sample 11 was composed of tablet triturates containing ¼ grain (0.016 gram) of santonin; 10 tablets were used as a sample. Sample 13 was composed of tablet triturates containing 1 grain (0.065 gram) of santonin; 2 tablets were used as a sample. Samples 5, 7, 9, 11 and 13 were commercial samples of santonin preparations.

Langer's Method (Modified)

Weigh out a sample equivalent to approximately 0.15 gram of santonin and extract the powdered sample with 10, 10, 10, 5, and 5 cc. portions of petroleum ether saturated with santonin (if the sample is fat-free this step may be omitted). Filter each portion of solvent with the aid of suction to complete dryness before following with another portion of fresh solvent through a Gooch crucible provided with an asbestos mat. Extract the residue in the solution flask and the crucible with 15, 10, 5, and 5 cc. of hot benzene, filtering each portion as before. Transfer the benzene extract to a tared flask, evaporate the solvent, and dry the residue to constant weight at 100°C. The weight of the santonin in the flask is equal to the weight of santonin in the sample. As a check, dissolve the residue in 25 cc. of aldehyde-free neutral alcohol by warming, neutralize, add 5 drops of phenolphthalein, and then add 25 cc. of 0.1 *N* KOH; digest this mixture on a water bath under a reflux for ½ hour and then titrate the hot solution with 0.1 *N* HCl, using 5 drops of phenolphthalein. Run a blank consisting of 25 cc. of aldehyde-free neutral alcohol and 25 cc. of the base in the same manner.

COMMENTS

C. O. Ewing.—The gravimetric determination seems to be the more reliable and the more direct one. There are chances that some inert material might go into solution along with the santonin, as the results from quite a number of the volumetric methods are lower than those from the gravimetric method. The whole method appears to be comparatively easy and free from manipulations that might cause error.

L. E. Warren.—I have carefully followed your directions concerning the use of hot benzene in the quantities prescribed and have afterwards subjected the residue in the crucible to extraction in a Bailey extractor, using further quantities of benzene. I found that there appears to be an incomplete extraction by your method.

L. Patricelli.—During extraction all the samples were placed over the steam bath after the first portion of benzene was added and allowed to boil a few minutes. The benzene used was kept on the steam bath between extractions. So I believe the trouble is that the solvent acts too slowly.

H. M. Burlage.—It was found early in the investigations that incomplete extraction was obtained when the lozenges and unground table triturates were extracted with hot benzene. Accordingly these preparations were triturated in a glass mortar and the powder thus obtained was then extracted with hot benzene. This information was discovered too late to transmit to all the collaborators.

DISCUSSION

It will be noted that incomplete results are presented for the volumetric determinations.

The weights of commercial preparations (lozenges and tablet triturates) showed considerable variation for the respective samples used. These variations may be beyond the control of the manufacturer, but they are worthy of investigation. The variations in weights range from

TABLE 2.

Collaborative studies by Langer's method (modified)
(In terms of weights of santonin found)

NO. OF SAMPLE	WEIGHT OF SAMPLE		CALCULATED WEIGHT OF EACH TABLET		STATED QUANTITY OF SANTONIN IN EACH TABLET	SANTONIN PER TABLET (GRAVIMETRIC)				SANTONIN PER TABLET (VOLUMETRIC)	
	B	P	B	P		B	E	W	P	B	E
	gram	*gram*	*gram*	*gram*		*gram*	*gram*	*gram*	*gram*	*gram*	*gram*
(5)	6.2358	6.3860	1.2472	1.2772	½ grain	0.0378	0.0366−	0.0402	—	0.0320+	0.0369
Lozenges	6.5670	6.4563	1.3134	1.2915	0.0325gm.	0.0393	0.0360+	0.0391			0.0359
							0.0362+				0.0360
					Average	0.0385+	0.0363	0.0397−			0.0363−
					Grand Average	0.0382+				0.0342	
(7)	1.9342	1.9288	0.0967	0.0964	1/10 grain	0.0068	0.0068	0.0067	0.0066+	0.0069	0.0068−
20 Tablets	1.9449	1.9252	0.0972	0.0963	0.0065 gm.	0.0069	0.0068−	0.0068+	0.0060−	0.0068	0.0068−
							0.0067				0.0067
					Average	0.0068+	0.0067+	0.0068−	0.0063	0.0068+	0.0068−
					Grand Average	0.0066+				0.0068	
(9)	0.3441	0.3219	0.1720	0.1610	1 grain	0.0693	0.0630	0.0629	0.0661−	0.0717	0.0629
Tablets	0.3205	0.3275	0.1602	0.1638	0.0650gm.	0.0654	0.0633−	0.0622	0.0600	0.0641	0.0655
	0.3251		0.1626			0.0646	0.0628			0.0691	0.0621−
					Average	0.0664−	0.0630+	0.0626−	0.0630+	0.0684	0.0635
					Grand Average	0.0637+				0.0659	
(11)	0.9479	0.9358	0.0948−	0.0936	¼ grain	0.0169	0.0171	0.0166+	0.0155+	0.0169	0.0162+
10 Tablets	0.9600	0.9859	0.0960	0.0986	0.0162+	0.0169	0.0167	0.0161+	0.0169−	0.0160	0.0160+
	0.9470		0.0947		(gm.)	0.0166	0.0168	0.0166+		0.0157	0.0167−
					Average	0.0168	0.0169−	0.0163−	0.0162−	0.0162	0.0163
					Grand Average	0.0165				0.0162+	
(13)	0.1505	0.1597	0.0752	0.0799	1 grain	0.0616−	0.0647−	0.0601	0.0639	0.0670	0.0651
2 Tablets	0.1581	0.1516	0.0791	0.0758	0.0650 gm.	0.0648−	0.0643−	0.0606	0.0607	0.0690	0.0640
	0.1537		0.0768			0.0633+	0.0648	0.0609		0.0620	0.0636
					Average	0.0632+	0.0446	0.0605+	0.0623	0.0660	0.0642+
					Grand Average	0.0626+				0.0659	

0.0092 gram (sample 13) to 0.3312 gram (sample 5) for samples whose weights range from 0.1505 to 6.5670 gram (sample 5). The effects of these weights are somewhat noticeable in the various amounts obtained for the total amount of santonin in the samples. Fortunately these variations are not sufficient to greatly affect the amount found in each lozenge and tablet. Much more noticeable are these weight variations in the determinations of the percentage of santonin in the samples where weights must be considered in the establishment of the results.

A study of the table shows the method to be applicable to non-fatty powder mixtures of santonin. Volumetric results check with the gravimetric data reasonably well although the use of the volumetric method lengthens the time of determination considerably. The method apparently does not give accurate results for fatty mixtures, since results are low and in considerable variance.

In answer to the comment made by Warren on extraction with benzene, it is apparent that continued extraction with hot benzene gives high results. If the material is powdered before extraction, the quantities of solvent mentioned seem to be sufficient.

General averages obtained by gravimetric and volumetric methods for the amount of santonin in each tablet check quite well.

SUMMARY

Encouraging results are obtained by the modified method given. Because of the time consumed, the volumetric method as a check is not advisable.

Methods of assay of santonin in the santonin-producing drugs are now being studied.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method submitted be adopted for the assay of santonin in mixtures and tablets of non-fatty nature and tentatively for the assay of santonin in fatty mixtures.

(2) That the present study of methods for the assay of santonin in the crude drugs being conducted by the associate referee be subjected to a collaborative investigation.

No report on ether was given by the associate referee.

REPORT ON BIOASSAY OF DRUGS

By Wm. T. McCloskY (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The Pharmacological Laboratory of Food and Drug Administration has completed an extensive study on the pharmacology of ergot, with

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 51 (1931).

particular respect to its biological assay and standardization. The results are presented in a series of nine articles published in the November, 1929, to July 30, 1930, numbers of the *Journal of the American Pharmaceutical Association.*

Among the outstanding features of the work are:

(1) *A Method for the Preparation of a Purified Fluidextract of Ergot.* The extraction of ergot by the method of U.S.P.X. yields a fluidextract containing practically the whole of the available alkaloids and amines present in the parent drug, together with much inert extractive matter. The amines are not responsible for any of the desirable clinical effects of ergot, and on account of their powerful local action on the smooth muscle of the stomach may be partly responsible for the transitory gastric disturbances (vomiting, nausea, etc.) which occasionally follow the administration of fluidextract of ergot. Moreover, the presence of amines in fluidextracts interferes with the assay of the specific alkaloids by certain of the current methods. Therefore, attempts were made to prepare a purified fluidextract as free as possible from amines and inert matter. The method finally adopted was based on the complete solubility of the amines, and relative insolubility of the specific alkaloids, in water. Since practically all samples of ergot are more or less acid, it was necessary to percolate first with a mild alkali, which afterwards was washed out with water, and of a number tried, sodium bicarbonate was found most suitable. The method is given in the 1930 revision of *Methods of Analysis.*

(2) *A study of the Broom and Clark method of assay of ergot preparations, with certain modifications.* It is desired to include this method in a recommendation as an alternative method for the assay of ergot preparations. The details of the original method and the modifications are given in the 1930 revision of *Methods of Analysis.*

The method for the standardization of ergot on the isolated rabbit's uterus depends upon the peculiar property of the specific alkaloid which enables it to paralyze the motor action of adrenalin on the plain muscle of the uterus of the rabbit.

Occasionally the musculature of the uterus of a rabbit, when suspended in the bath, does not respond in the usual way to adrenalin. It will be seen that on the addition of adrenalin (before doses of ergot extracts are added) a tonic contraction is not produced. Having contracted, the contraction is not maintained, but relaxation occurs at once before the Locke-Ringer solution is changed. A muscle which behaves in this way may give misleading results, and in general should not be used. Provided these different points are heeded, the discrimination which may be obtained is sometimes surprisingly great, and observations on different pairs of strips show remarkable consistency.

In the course of an assay single observations may be made which are seriously misleading. Thus a single observation may indicate a

strength about 0.1 per cent where the true value is 0.067 per cent; or an extract may appear greater than 0.15 per cent where the true value is 0.11 per cent. These aberrant results make it important to repeat each observation if the difference observed is great.

The uterine horns of different animals vary considerably in the discrimination which they will give. Occasionally pieces of muscle will not distinguish between less than a 50 per cent difference of dose.

A study of the rabbit uterus method led to the following conclusions: The contractions produced by adrenalin (epinephrine) should be constant and appreciable but sub-maximal. It is important to allow the same time interval between successive doses of adrenalin. The doses of ergot should produce a degree of inhibition between 50 and 75 per cent. An assay is complete only when a quantity of an unknown preparation which will produce the same inhibition as a definite amount of the standard preparation has been determined. The experimental error of the method is about 10 per cent.

The work on ergot by the Pharmacological Laboratory has been confirmed by commercial and research laboratories.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method outlined for the preparation of a purified fluidextract of ergot be made a tentative method.

(2) That the method for the assay of ergot on the isolated rabbit's uterus be made a tentative method.

(3) That the cat-eye method for the assay of mydriatics and myotics elaborated by the former associate referee be made on official method.

REPORT ON EPHEDRA

By C. K. GLYCART (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

During the past three years the work on ephedra and its alkaloids included a method for the assay of the crude drug[2] and a quantitative determination of ephedrine in tablets[3] by Paul and Glycart. A method for ephedrine in inhalants was added this year in accordance with the recommendation that ephedrine in pharmaceuticals be further studied.

The formulas of ephedrine inhalant sprays usually contain 1 per cent of ephedrine alkaloid dissolved in mineral oil and cottonseed oil in combination with small quantities of menthol, thymol, camphor, and aromatic oils.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 51 (1931).
[2] *This Journal*, 12, 291 (1929).
[3] *Ibid.*, 13, 329 (1930).

The sample that was submitted to the collaborators was prepared by dissolving the following ingredients:

	grams
Ephedrine alkaloid	4
Menthol	0.4
Eucalyptus oil	2.0
Cinnamon oil	1.0

in sufficient mineral oil to make 300 grams of solution. The ephedrine alkaloid was prepared from a commercial product of ephedrine hydrochloride by extraction of the liberated alkaloid with ammoniacal ether solvent. After evaporation of the solvent the residue was dissolved in water and the extraction with ether was repeated. The residue was stirred until white crystalline particles were formed. After desiccation the melting point was determined to be 39°C. The test for chlorides was negative. Ephedrine, by direct titration, equaled 99.87 per cent.

EPHEDRINE INHALANTS

REAGENTS

(a) *Sulfuric acid.*—2 per cent solution.

(b) *Washed ether.*—Shake equal volumes of ethyl ether and water in a separatory funnel and discard the aqueous layer.

(c) *Strong ammonia.*

(d) *Bromthymol blue indicator.*—0.04 per cent alcoholic solution.

(e) *Sulfuric acid.*—0.02 *N* solution.

(f) *Distilled water.*—Free from carbon dioxide.

(g) *Sodium hydroxide.*—0.02 *N* solution, free from carbonates.

DETERMINATION

Weigh accurately into a small tared beaker, 5–10 grams of the sample and add 10 cc. of 2 per cent sulfuric acid. Stir the mixture, allow to stand about 15 minutes, then transfer to a small separatory funnel (automatic extractor optional), rinsing the beaker with small portions of ether. Shake gently and transfer the acid layer to a second separatory funnel. Shake with three successive 10 cc. portions of the sulfuric acid reagent, rinsing the beaker with ether each time. (Test for complete removal of alkaloid.)

Neutralize the combined acid solution with strong ammonia, then add 5 cc. in excess. Extract the solution with 30 cc. of washed ether. Transfer the aqueous layer to a second separatory funnel. Wash the ether extraction with 1 cc. of water, adding the washings to the main aqueous solution. Swirl the ether in order to remove water adhering to the side of the separatory funnel. After all the water has been removed, filter into an Erlenmeyer flask through a pledget of cotton wet with ether inserted in a small funnel. Repeat the extraction with liberal portions of ether at least four times, or until the alkaloid is removed completely, washing each portion with the same 1 cc. of water. Evaporate the ether to a volume of 10 cc. on a steam bath with moderate heat by the aid of a current of air.

Titration Procedure No. 1: Remove from the heat and finish evaporation at room temperature before the fan. Dissolve the alkaloidal residue in 2 cc. of neutral alcohol and dilute with about 40 cc. of carbon-dioxide-free water. Titrate with 0.02 *N* sulfuric acid to yellow color with bromthymol blue indicator, using standard indicator, *p*H 6.0.

Titration Procedure No. 2: Remove from the bath and add bromthymol blue indicator and a measured excess of 0.02 *N* sulfuric acid. Add about 40 cc. of carbon-dioxide-free water, cover with a watch-glass, return to the steam bath in order to dissolve the alkaloid adhering to the sides of the flask, and evaporate all the ether. Titrate the excess acid with 0.02 *N* sodium hydroxide, using standard indicator, *p*H 6.0, for comparison. 1 cc. of 0.02 *N* acid = 0.0033 gram of ephedrine alkaloid.

Results on ephedrine inhalant.

COLLABORATOR	EPHEDRINE	
	Titration Procedure No. 1	Titration Procedure No. 2
	per cent	*per cent*
Walter Hoover Eli Lilly & Co. Indianapolis, Ind.	1.364	1.363
J. B. Williams Parke Davis & Co. Detroit, Mich.	1.30 1.29 1.29	1.35 1.34 1.33
H. McCausland The Abbott Laboratories North Chicago, Ill.	1.28	1.22
Frank C. Sinton Food and Drug Adm. Chicago, Ill.	1.29 1.27	1.33
C. K. Glycart	1.31	1.34

COMMENTS OF COLLABORATORS

Walter Hoover.—The two methods for titration checked very closely and neither method gave any difficulty, although the residual method of heating on a water bath with an excess of acid and titrating back with standard alkali solution is preferred. The use of the automatic extractor for ephedrine products as a means of insuring more complete extraction is recommended. A determination made by using the automatic extractor on the A.O.A.C. sample gave 1.396 per cent. A determination made on the A.O.A.C. sample following titration procedure No. 2, in which methyl red was used as the indicator, yielded 1.369 per cent.

In addition to the data given on the inhalant submitted by your Department, we include the following figures for an inhalant containing 1 per cent of ephedrine: Titration procedure No. 1, 0.9906; Titration Procedure No. 2, 0.9902.

These figures are given simply to show the close checks that are obtained by the two titration procedures. As there was not a sufficient amount of the sample submitted to permit as many assays as we should like to have made, we used the additional sample.

J. B. Williams.—The sample was also assayed by the following slightly modified method: 5 grams was transferred to the separator with the aid of about 25 cc. of ether and shaken out with 10, 5, 5, 5, and 5 cc. portions of 2 per cent sulfuric acid.

A. The acid solution was made alkaline with sodium hydroxide and shaken out with six 25 cc. portions of ether, a measured excess of 0.1 *N* acid was added, and the ether was evaporated.

B. The acid solution was made alkaline with ammonia and shaken out with six

25 cc. portions of ether, the ether solution was evaporated to about 5 or 10 cc., a measured excess of 0.1 *N* acid was added, and evaporation of ether was completed. The excess acid was titrated with 0.02 *N* NaOH. Methyl red indicator was used.

A = 1.37 per cent ephedrine.
B = 1.38 per cent ephedrine.

F. C. Sinton.—The end points were ascertained without difficulty by aid of a bromthymol blue color comparator, *p*H 6.0.

It is suggested that in titration procedure No. 1 provision be made for washing down ephedrine adhering to the sides of the beaker due to creeping of the residue. I prefer titration procedure No. 2 because it eliminates possible loss through evaporation of ephedrine.

DISCUSSION

The lower results obtained by titration procedure No. 1 are evidently due to loss of alkaloid by volatilization on evaporation to dryness. The results by titration procedure No. 2, with one exception, are in close agreement and also correspond to the theoretical figure, 1.333 per cent of ephedrine. The addition of a measured volume of 0.02 *N* sulfuric acid to the ether before complete evaporation tends to eliminate possible loss of alkaloid.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for ephedra assay, now tentative, be amended by deleting titration procedure No. 1, and that the method, so amended, be adopted as official, first reading, with a view to final action.

(2) That the quantitative method for the determination of ephedrine in tablets,[2] be amended by deleting near the top of p. 330, the statements beginning with "see discussion," and ending with "convenient for a control," and substituting therefore procedure No. 2 as described in this report; and that the method so amended be made tentative, with a view to adoption as official.

(3) That the qualitative color test by copper sulfate and also the melting point determination[3] be made tentative with a view to adoption as official.

(4) That the method for the determination of ephedrine in inhalants described in this report, but omitting titration procedure No. 1, be made tentative with a view to adoption as official.

REPORT ON THYMOL

By LESLIE HART[4] (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

A progress report on the determination of thymol in *Liquor Antisepticus* (National Formulary) was submitted to the association in 1929.[5]

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 52 (1931).
[2] *This Journal*, 13, 329 (1930).
[3] *Ibid.*, 330.
[4] Presented by C. K. Glycart.
[5] *This Journal*, 13, 332 (1930).

Further experimental work done by the associate referee has resulted in methods for the determination of thymol in acidic solutions, such as *Liquor Antisepticus*, and in alkaline solutions, such as *Liquor Aromaticus Alkalinus*. These methods were submitted to collaborators, and their results are given in this report.

A review of the literature revealed no methods of assay applicable to alcoholic-aqueous solutions of thymol in the presence of essential oils or other volatile, ether-soluble constituents. It was therefore necessary to formulate methods to determine small quantities of thymol in the presence of these interfering substances.

It was observed by Sherk[1] that thymol may be removed from aqueous alkaline solution by successive extractions with ethyl ether. This statement was corroborated by experiment, and the fact was utilized in the separation of thymol from interfering constituents.

The basis of the methods finally adopted is the removal of alcohol and the simultaneous saponification of methyl salicylate by evaporation on the steam bath. Extraction with petroleum ether removes menthol and essential oils, and further extraction with ethyl ether removes thymol. The ethereal solution is then evaporated in the presence of alcoholic potash solution, the residue is taken up with hot water and acidified, and the thymol is titrated with 0.1 *N* bromine solution, as outlined in the report on assay of U. S. P. thymol.[2]

A brief description of preliminary experiments leading to the adoption of the various steps of these methods may be of interest.

EXTRACTION OF THYMOL FROM ALKALINE SOLUTION WITH ETHER

A 50 cc. aliquot of an aqueous solution containing 5 per cent sodium hydroxide and 0.1 gram of thymol was extracted four times with 25 cc. portions of ethyl ether. The residual thymol in the alkaline solution was then determined. This was found to be 0.0040 gram and 0.0020 gram, on duplicate determinations.

LOSS OF THYMOL BY EVAPORATION FROM ALKALINE SOLUTION

To another 50 cc. of the thymol solution (containing 0.1 gram of thymol) were added 25 cc. of alcohol and 5 cc. of 50 per cent sodium hydroxide solution, and the mixture was evaporated on the steam bath to 50 cc. volume. The amount of thymol in the solution was then determined and found to be 0.1003 gram and 0.0997 gram on duplicate determinations, showing no loss in thymol.

One-tenth gram of thymol was dissolved in 50 cc. of ether, 5 cc. of alcoholic potash solution (40 grams per liter) was added, and the mixture was evaporated to a volume of 5–6 cc. on the steam bath. Evaporation

[1] *Am. J. Pharm.*, **93**, 115, 207 (1921).
[2] *This Journal*, **12**, 54, 296 (1929).

was aided by a current of air from an electric fan. The determinations of thymol in the residues revealed no appreciable loss, as shown below.

	gram
(1)	0.0975
(2)	0.0985
(3)	0.0947
(4)	0.0990

EFFECT OF ESSENTIAL OIL CONSTITUENTS ON THE SEPARATION OF THYMOL

Two mixtures were prepared as follows: (A) solution containing 0.1 gram of thymol, 0.6 gram of sodium benzoate, 2.5 grams of boric acid and 30 grams of alcohol per 100 cc.; (B) solution of 5 cc. of eucalyptol, 1.2 cc. of methyl salicylate, 1 gram of menthol in sufficient 97 per cent alcohol to make 100 cc. Five cc. of solution B represents the amount of each of these constituents found in 50 cc. of *Liquor Antisepticus*.

To 50 cc. of solution A containing 0.050 gram of thymol, varying amounts, 5–15 cc., of solution B were added, then 5 cc. of 50 per cent sodium hydroxide solution. The mixture was dealcoholized on the steam bath, cooled, and extracted twice with petroleum ether, and the aqueous layer was extracted five times with ethyl ether. The thymol was determined in the ethyl ether extract by titration with 0.1 *N* bromine. The results follow:

Solution B present	Thymol found
cc.	*gram*
5	0.0490
5	0.0500
10	0.0475
15	0.0488

LIQUOR AROMATICUS ALKALINUS

A solution of *Liquor Aromaticus Alkalinus*, following the formula given in the National Formulary, 4th ed., with the exception of the omission of thymol from the formula, was prepared and filtered through magnesium carbonate. Five-tenths gram of thymol was then dissolved in the filtrate, and the mixture was made up to 1000 cc. with alcohol and water. The final alcoholic content was about 15 per cent.

One hundred cc. aliquots were analyzed, first by the method outlined in the section "Effect of essential oil constituents"; second, the mixture was acidified with hydrochloric acid, made alkaline with sodium hydroxide solution, and analyzed for thymol by the same method. Results were variable and quite low. These low results are apparently due to the presence of glycerol. Various solutions, differing in their glycerol content

and in the amount of 50 per cent sodium hydroxide solution added, were assayed for thymol according to the method given under the heading "Effect of essential oil constituents." This method calls for 5 extractions of the alkaline solution with ethyl ether, which was found to be sufficient to extract the thymol from preparations of the *Liquor Antisepticus* type. The results follow:

SOLUTION ASSAYED	GLYCEROL PRESENT	50% NAOH ADDED	NO OF ETHER EXT'NS	THYMOL—GRAMS PER 100 cc	
				Present	Found
	cc.	*cc.*			
100 cc contains 2.5 grams of boric acid	none	5	5	0.100	0.099
100 cc contains 2.5 grams of boric acid	15	5	5	0.100	0.091
100 cc contains 2.5 grams of boric acid	10	5	9	0.100	0.098
Liquor Aromaticus Alkalinus	none	5	5	0.050	0.048
Liquor Aromaticus Alkalinus	none	5	6	0.050	0.048
Liquor Aromaticus Alkalinus	10	5	6	0.050	0.044
Liquor Aromaticus Alkalinus	10	5	5	0.050	0.043
Liquor Aromaticus Alkalinus	10	5	7	0.050	0.048
Liquor Aromaticus Alkalinus	10	5	9	0.050	0.049
Liquor Aromaticus Alkalinus	10	2	5	0.050	0.032
Liquor Aromaticus Alkalinus	10	2	8	0.050	0.032
Liquor Aromaticus Alkalinus	10	2.5	6	0.050	0.026
Liquor Aromaticus Alkalinus	10	(Just alk. to M.O.)	5	0.050	0.036

To other 100 cc. aliquots 5 cc. of 50 per cent sodium hydroxide were added, the mixture was dealcoholized and extracted twice with petroleum ether, and a stream of carbon dioxide was passed through the aqueous layer for 15 minutes. The thymol was then extracted with ethyl ether, alcoholic potash solution was added to the ether extract, the ether was evaporated, and the amount of thymol was determined. Triplicate results show that practically all the thymol was recovered.

gram per 100 cc.

(1) 0.0473
(2) 0.0479
(3) 0.0489

As a result of this experimental work, methods for the determination of thymol in (a) *Liquor Antisepticus* and similar acidic solutions and (b) *Liquor Aromaticus Alkalinus* and similar alkaline solutions, together with solutions of *Liquor Antisepticus* containing 0.10 gram of thymol per 100 cc., and *Liquor Aromaticus* containing 0.05 gram of thymol per 100 cc. were submitted for collaborative study.

THYMOL IN MOUTH WASHES AND SIMILAR PREPARATIONS

There are two such preparations containing thymol recognized by the National Formulary: (1) *Liquor Antisepticus*, which is acid in reaction and (2) *Liquor Aromaticus Alkalinus*, which is alkaline. These preparations require slightly different treatment. In addition there are many proprietary preparations on the market more or less similar to these preparations.

REAGENTS

(a) *0.1 N bromine solution.*[1]—Dissolve 3 grams of potassium bromate and 50 grams of potassium bromide in water. Dilute to one liter with water at 20°C. 1 cc of 0.1 *N* solution = 0.003753 gram of thymol. This may be standardized by titration with 0.1 *N* thiosulfate, or against U.S.P. thymol.[2]

(b) *Hydrochloric acid.*—Concentrated.

(c) *Methyl orange.*—0.1 gram to 100 cc. of water.

(d) *Sodium hydroxide solution.*—50%.

(e) *Sodium hydroxide solution.*—5%.

(f) *Alcoholic potash solution.*—40 grams per liter.

(g) *Carbon dioxide.*

(h) *Petroleum ether.*

(i) *Ethyl ether.*

DETERMINATION

If the alcoholic content is not known, make a preliminary determination of alcohol.

Transfer an aliquot containing 0.05–0.10 gram of thymol to a platinum or porcelain evaporation dish. Test the sample with litmus paper. If alkaline in reaction, add 5 cc. of 50 per cent sodium hydroxide solution; if acid, neutralize with the 50 per cent sodium hydroxide solution and add 5 cc. in excess. Dealcoholize, by removing by evaporation on the steam-bath before an electric fan, a volume slightly in excess of the volume of alcohol present.

Transfer the solution to a 125 cc. separatory funnel, wash out the dish with 20 cc. of water, and add the washings to the liquid in the funnel. Extract twice with petroleum ether, using 20 cc. each time. Wash the combined extracts once with 5–10 cc. of 5 per cent sodium hydroxide solution and add washings to the aqueous layer in the separatory funnel.

The procedure from this point varies slightly, depending upon whether glycerol was present in the original sample.

PROCEDURE FOR PRODUCTS IN WHICH GLYCEROL IS ABSENT

For products which originally contained glycerol proceed as follows:

Extract the aqueous alkaline solution and washings remaining from the petroleum ether extraction, which contains the thymol, together with sodium salts of boric, benzoic and salicylic acids, with ethyl ether, making five extractions (20, 15, 15, 10, 10 cc.).

Combine the ether extracts, transfer to a 250 cc. glass-stoppered Erlenmeyer flask, add 5 cc. of recently prepared alcoholic potash solution, and evaporate most of the ether, using steam bath and electric fan. Do not evaporate entirely to dryness but leave from 5 to 8 cc. residue. To this residue add 75 cc. of hot water (80–90°C.) and 10 cc. of concentrated hydrochloric acid.

[1] *Methods of Analysis, A.O.A.C.*, 1925, 381.

[2] *This Journal*, 12, 54 (1929).

Immediately run in 1–3 cc. less than the theoretical amount of 0.1 *N* bromine solution, swirling the contents of the flask constantly. Add 2 drops of methyl orange solution and titrate slowly with bromine solution, shaking vigorously after each addition. When the red color of the methyl orange is bleached add two drops of the titrating solution, stopper, shake vigorously for 10 seconds, add one drop of methyl orange solution, and again shake vigorously for 10 seconds. Continue the addition of bromine solution, 2 drops at a time, and shake after each addition, until the red color disappears. Then add one drop of methyl orange solution, shake vigorously, and if the red color does not disappear, repeat the alternate addition of two drops of bromine solution and one drop of methyl orange solution, shaking after each addition, as directed above, until the red color disappears. 1 cc. of 0.1 *N* bromine = 0.003753 gram of thymol.

If the theoretical amount of thymol present is not known, add two drops of methyl orange solution and titrate slowly, swirling constantly during the addition of bromine solution until the red color is bleached. Then continue according to the method outlined, beginning at the phrase: "Add two drops of the titrating solution, stopper, and shake vigorously"

CAUTION: *Both the evaporation of alcohol and the later evaporation of ether must be done very carefully in order to avoid loss of thymol by volatilization.*

PROCEDURE FOR PRODUCTS CONTAINING GLYCEROL

For products which originally contained glycerol proceed as follows:

Pass a stream of carbon dioxide for 15 minutes through the alkaline aqueous layer remaining after the petroleum ether extraction. Extract five times with ethyl ether, using 20, 20, 15, 15, and 10 cc.

Combine the ether extracts, and continue the determination from this point as described above in the method for products in which glycerol is absent, beginning with the paragraph "Combine the ether extracts, transfer to a 250 cc glass-stoppered Erlenmeyer flask" Test for complete extraction with ethyl ether by extracting the aqueous, alkaline residue with 20 cc. of ether and determining the thymol extracted, if any.

COMMENTS OF COLLABORATORS

Liquor Antisepticus Method

L. S. Crosby.—We are of the opinion that better results could be secured if the bromate solution were added in excess at once and titrated back as in Method B (Jr. A.O.A.C. XII: 54.)

W. F. Kunke.—I think it would be advisable to specify the use of a *glass-stoppered* 250 cc. Erlenmeyer, and also the *range of temperature* of the 75 cc. of hot water added. (These suggestions have been incorporated into the method by the referee.) Mr. Kunke further states: . . . "the procedure appears sound and works smoothly. More concordant results may be obtained if the last evaporation is not carried on below the 5 cc. minimum value."

N. L. Knight.—Details of separation and titration of the thymol must be followed exactly as stated in the method in order to ensure concordant results.

R. D. Stanley.—Concordant results may be expected if proper precautions as stated in the method are observed.

Liquor Aromaticus Alkalinus Method

W. F. Kunke.—I would suggest that you add: "test for complete extraction of the carbonated aqueous layer by extracting with 20 cc. of ether, and determining the thymol, if any." (This suggestion has been incorporated by the referee.)

RECOMMENDATIONS[1]

It is recommended—

(1) That the methods for the determination of thymol in *Liquor Antisepticus*, and *Liquor Aromaticus Alkalinus* be adopted as tentative methods.

(2) That no further work be done on thymol preparations by the A.O.A.C. at this time.

TABLE 1.

Results of collaborators on sample of Liquor Antisepticus.

(0.1 gram of thymol per 100 cc.)

COLLABORATOR	NO. OF ASSAYS	GRAMS OF THYMOL PER 100 CC. *Maximum*	*Minimum*	*Average*
Leslie Hart	10	0.102	0.094	0.0987
R. D. Stanley U.S. Food and Drug Laboratory St. Louis, Mo.	2	0.102	0.101	0.1015
N. L. Knight U.S. Food and Drug Laboratory St. Louis, Mo.	4	0.097	0.093	0.095
W. F. Kunke U.S. Food and Drug Laboratory Chicago, Ill.	3	0.100	0.099	0.0993
L. S. Crosby United Drug Co. Boston, Mass.	2	0.108	0.101	0.1045
L. S. Crosby United Drug Co. Boston, Mass.	1	0.0983 (back titration with thiosulfate).		

Average of 21 determinations by 5 analysts: 0.0989 gram

TABLE 2.

Results of collaborators on sample of Liquor Aromaticus Alkalinus.

(0.05 gram of thymol per 100 cc.)

COLLABORATOR	NO OF ASSAYS	GRAMS OF THYMOL PER 100 CC. *Maximum*	*Minimum*	*Average*
Leslie Hart	6	0.0496	0.0490	0.0495
R. D. Stanley	2	0.0491	0.0491	0.0491
W. F. Kunke	2	0.0492	0.0490	0.0491
N. L. Knight	1			0.0488

Average of 11 determinations by 4 analysts: 0.0493 gram.

Due to the fact that this method is but a modification of the method described for *Liquor Antisepticus* three collaborators were deemed sufficient to test the accuracy of the method.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 52 (1931).

REPORT ON MENTHOL

By F. L. Elliott (U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

It was recommended that the method for the determination of menthol be further studied.

Since the method submitted to collaborators in 1928 was found to give fairly satisfactory results, and suggested changes did not result in any improvements, the original method was sent to other collaborators with the view to recommending it as a tentative method for the determination of menthol.

A sample of U.S.P. menthol was submitted; the results, expressed in percentage, are as follows:

W. J. Rice Eli Lilly & Co. Indianapolis, Ind.	100 36	100.18	100.30	100.26
J. P. Snyder Norwich Pharmacal Co. Norwich, N.Y.	100.00 (a) 100.24 (b)			
E. O. Eaton U.S. Food and Drug Adm. San Francisco, Calif.	100.3			
H. H. Mottern Bur. Chemistry and Soils U.S. Dept. Agr. Washington, D.C.	100.2			
Wm. F. Kunke U.S. Food and Drug Adm. Chicago, Ill.	99.7	99.9		
T. N. Bennett U.S. Food and Drug Adm. New York, N.Y.	100 9	101.3		
R. L. Herd U.S. Food and Drug Adm. Baltimore, Md.	99.26	99.47	99.80	

COMMENTS OF COLLABORATORS

J. P. Snyder.—The method gives slightly high results, but on the other hand it does show a high degree of accuracy, at least sufficient for general laboratory purposes.

H. H. Mottern.—Method quite satisfactory for U.S.P. menthol. The melting point should also be determined.

Wm. F. Kunke.—Suggest the use of 6.25 grams of monohydrated sodium carbonate instead of diluting the U.S.P. test solution.

RECOMMENDATION[1]

The results of collaborators this year are again quite satisfactory, and the method is recommended as a tentative method.

SUPPLEMENTARY REPORT

(Results received from collaborators after the report was submitted.)

	PER CENT	PER CENT
A. Barol Sharp and Dohme Baltimore, Md.	98.10 (1) 97.42 (2) 99.99 (3)	
Chas. E. Vanderkleed Robert McNeil Philadelphia, Pa.	100.12	100.12
F. A. Rotondaro The Zemmer Co. Pittsburgh, Pa.	102.5	

COMMENTS

A. Barol.—Analyses No. 1 and No. 2: Acetylized oil in contact with wash solution a little longer than necessary (due to moving laboratory). *Analysis No. 3:* Washings removed after standing one hour. Three washings were required.

F. A. Rotondaro.—Average of five determinations. The method gives high results. A good control method, as individual results are fairly consistent.

REPORT ON BROMIDES—CHLORIDES

By N. E. FREEMAN[2] (U. S. Food and Drug Administration, New Orleans, La.), *Associate Referee*

At the 1928 meeting of this association H. Wales[3] reported on the Winkler method[4] for the determination of bromides in the presence of chlorides. Subcommittee B recommended that this method be studied collaboratively, that the applicability of potentiometric methods be studied, and that the problem of the separation by chemical means of the three halogens be studied.

This report is therefore divided into three parts according to the above recommendations.

I.

Unfortunately it was impossible to duplicate the former associate referee's work on this method. The titration was exceedingly tedious owing to the period of boiling required after each small addition of the

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 52 (1931).
[2] Presented by R. S. Roe.
[3] *This Journal*, 12, 302 (1929).
[4] *Z. angew. Chem.*, 28, I, 477 (1915).

permanganate. Even then the end point was very indefinite due to the precipitation of manganese peroxide as the end point was approached.

For this reason a large portion of the available time was spent in attempting to modify the method so that a sharp end point could be obtained. The following method seemed to give quite concordant results, and it was therefore submitted to the collaborators.

Distillation Method

DETERMINATION

Weigh a quantity equivalent to about 0.3 gram of KBr and introduce into a 500 cc. distilling flask. Add 20 cc. of H_2SO_4 (1+3) and 200 cc. of H_2O. Connect the distilling flask with a condenser which dips into a solution containing 1 gram of $NaHSO_3$ and 5 cc. of the dilute H_2SO_4 in a volume of 100 cc. Insert a one-hole rubber stopper carrying a dropping funnel and heat the solution to boiling. Add drop by drop while boiling a 1.5% $KMnO_4$ solution until a pink color persisting for 1 minute is obtained. Continue to boil until at least 100 cc. has distilled over.

Boil the solution in the receiver until free from SO_2. Add 10 cc. more of dilute H_2SO_4 and 50 cc. of 0.1 *N* $KMnO_4$. Boil for 1 minute and aspirate with filtered air for ½ hour to remove Br. Add just enough 0.1 *N* $(COOH)_2$ to dissolve the precipitated MnO_2 and titrate this slight excess of $(COOH)_2$ with the 0.1 *N* KMO_4. The number of cc. of 0.1 *N* $KMnO_4$ used multiplied by 0.0119 equals the weight of KBr.

DIRECT TITRATION

Weigh a quantity of sample equivalent to not more than 0.3 gram of KBr into a 500 cc. Erlenmeyer flask and add 150 cc. of H_2O, 10 cc. of the $MnSO_4$ solution, and 15 cc. of dilute H_2SO_4 (1+3). Add slowly, with constant agitation, 50 cc. of 0.1 *N* MNO_4 and proceed as above, beginning with "Boil for 1 minute." (A Folin aspirator tube containing a small plug of cotton works very nicely for the aspiration.)

From the collaborative results and those obtained by the associate referee, it was apparent that the samples lacked uniformity, due, undoubtedly to the difficulty of obtaining the same degree of fineness in all the ingredients. Therefore, liquid samples were prepared and analyzed by E. C. Deal of this laboratory and the associate referee. The following results were obtained.

		KBr per 25 cc.	Recovered
		gram	*per cent*
	Sample 1		
By distillation	N.E.F.	0.2836	102.0
		0.2854	102.9
	E.C.D.	0.3207	115.5
		0.3254	117.1
	Sample 1		
Direct	N.E.F.	0.2818	101.4
		0.2824	101.9
	E.C.D.	0.3052	110.0
	Sample 2		
Direct	N.E.F.	0.2873	103.5
		0.2889	104.0
	E.C.D.	0.3100	111.6

Samples 1 and 2 each contained 0.2777 gram of KBr per 25 cc. of solution. No. 2 in addition contained 0.3125 gram of KCl.

From the figures shown it is apparent that either this method should be further studied or another one be found.

II.

Only a limited amount of work was done on potentiometric methods, including the use of a silver electrode and titration with silver nitrate. Fairly accurate results were obtained between the iodide and bromide, but the other end points were not clearly defined, due undoubtedly to the fact that near the end of the bromide titration both silver bromide and silver chloride are precipitated. The only application of this method, therefore, seems to be the determination of iodine in the presence of either bromide or chloride.

III.

The literature consulted regarding the chemical means of separating the three halogens depends mainly upon indirect methods. Treadwell[1] reports a method based upon oxidizing the silver precipitate with dichromate, which converts the iodide to the iodate, and boiling off the liberated chlorine and bromine. The iodate is then reduced with sulfur dioxide, and the precipitate of silver iodide is filtered off and weighed. The residual silver is then precipitated as the iodide and weighed as such. Equations are then given for calculating the percentage of Cl, Br and I in the mixture.

The most promising direct method seems to be based upon the liberation of the iodine by $FeCl_3$ and distillation into an excess of standard thiosulfate solution, followed by liberating the bromine by oxidation with permanganate, as given in Part I. The chloride is then calculated by difference.

RECOMMENDATIONS[2]

It is recommended—

(1) That the permanganate method for bromides be further studied.

(2) That consideration of potentiometric methods for the halogens be dropped.

(3) That the method for the progressive removal and determination of the halogens, as outlined in part III, be studied.

[1] Treadwell-Hall, Analytical Chemistry, Vol II.

[2] For report of Subcommittee B and action of the association, see *This Journal*, 14, 52 (1931).

REPORT ON OIL OF CHENOPODIUM

By L. B. BROUGHTON (University of Maryland), *Associate Referee*

In accordance with the recommendations of Subcommittee B, the Paget method for the determination of ascaridole in oil of chenopodium was studied collaboratively. Samples were prepared and distributed for assays.

As the statement was made last year that carbon tetrachloride was often used with oil of chenopodium in the treatment of animals for worms, it was also requested that the study this year include samples of known percentage of ascaridole adulterated with this compound. Accordingly, samples containing 90.00 per cent of ascaridole, as determined by the U. S. P. and Paget assays, as well as by distillation, were prepared from a stock oil secured from the oil district in Maryland.

Sample No. 1.—The stock oil adulterated with carbon tetrachloride to yield a mixture containing 45 per cent of ascaridole.

Sample No. 2.—The stock oil and carbon tetrachloride, giving a mixture containing 67.5 per cent of ascaridole.

Sample No. 3.—A solution of stock oil and cineol, containing 60 per cent of ascaridole.

Sample No. 4.—A mixture of the stock oil and 95 per cent alcohol, containing 63.00 per cent of ascaridole.

Portions of each of these samples were submitted to collaborators, together with methods to be followed for both the U. S. P. and Paget methods. Table 1 gives the percentage of ascaridole reported in each sample by the U. S. P. method.

TABLE 1.

Ascaridole reported in samples (U. S. P. Method).

COLLABORATOR	SAMPLE NO 1	SAMPLE NO. 2	SAMPLE NO. 3	SAMPLE NO. 4
	Per cent	*Per cent*	*Per cent*	*Per cent*
1	44.00	70.00	81.50	92.50
2	55.00	72.00	90.00	96.00
3	59.00	76.90	83.00	95.00
	59.60	76.80	81.20	95.40
4	—	—	87.70	97.00
5	43.00	65.00	80.50	91.00
Average	52.15	72.15	83.98	94.55
Actually present	45.00	67.50	60.00	63.00

Variation is noted in the results reported in Table 1 for samples Nos. 1 and 2 containing carbon tetrachloride as an adulterant. This may be attributed to the fact that the density of the insoluble fraction is greater than the 60 per cent acetic acid solution, since to complete the assay by

the U. S. P. method it was necessary to invert the cassia flask in order to read the volume. The results show that the official method does not give an accurate measure of the ascaridole content under such conditions and is not applicable for the analysis of an oil containing an adulterant with a specific gravity greater than that of 60 per cent acetic acid.

Results on Samples 3 and 4 are also inconsistent. They show that the U. S. P. method is no measure for oils adulterated with cineol and alcohol. Cineol no doubt would never become a common adulterant, but it has been reported as one of the constituents in oil of chenopodium. Ethyl alcohol, on the other hand, offers possibilities as an adulterant, as mixtures of wormseed oil and alcohol, meeting the specifications of a standard oil can easily be prepared.

One of the collaborators[1] made the following comment on the present official method: "Aside from the discrepancies pointed out by Paget, Nelson and Broughton, the presence of an adulterant of greater specific gravity than the acid mixture is not provided for. It may be noted that the directions given are rather vague. The time of shaking, the period of standing, the advisability of centrifugalizing, and the method of reading the oil column are all points that are left to the individual worker."

The objections noted above are well founded and explain in a measure the inconsistent results reported.

ANALYSIS OF OIL SAMPLES BY THE PAGET METHOD

As reported to the association last year this method takes advantage of the oxidizing properties of the organic peroxide ascaridole and provides a means of measuring the active constitutent of an oil by titration with titanium trichloride and iron alum. The method is briefly outlined as follows:

> To a known weight of oil diluted with 96 per cent alcohol in a flask through which a current of carbon dioxide is passing, an excess of titanous chloride is added; the flask is then closed with a Bunsen valve, and its contents are heated almost to boiling for 1 or 2 minutes. About 1 cc. of a 5 per cent potassium thiocyanate solution is added, and the solution is titrated back with standard iron alum until a permanent faint red color is obtained. The amount of iron used, calculated in terms of titanous chloride, gives by difference the quantity of the titanous chloride oxidized. This is converted into ascaridole by the empirical factor 1.2770.

Following this method the four samples described above were examined. Table No. 2 presents the results reported by the collaborators.

An examination of the data in Table No. 2 shows that more uniform results were obtained by the use of the titanous trichloride method than by the official solubility assay. Collaborators, however, had some difficulty in checking their results, which was due, no doubt, to inexperience with the method. Average results, with the exceptions indicated, are

[1] Communication from W. F. Reindollar.

TABLE 2.

Ascaridole reported in samples (Paget Method).

COLLABORATOR	SAMPLE NO. 1	SAMPLE NO. 2	SAMPLE NO 3	SAMPLE NO. 4
	Per cent	*Per cent*	*Per cent*	*Per cent*
1	44.52	65.67*	65.22*	66.17*
	44.68	66.21	63.78	63.07
				62.35
2	43.40	63.48*	58.03	57.00*
	42.00*	66.50	60.30	63.60
3	—	—	—	—
4	—	—	—	—
5	44.93	66.72	60.86	62.49
	44.40	66.12	59.75	62.85
	44.30	68.26	59.96	63.18
Average	44.37	66.76	60.44	62.92
Actually present	45.00	67.50	60.00	63.00

* Not included in the average

within the limits of experimental error allowable in an analytical method. The method is not affected by carbon tetrachloride, cineol or alcohol when used as adulterants, and it gives a more accurate estimate of the ascaridole content of an oil than the official method.

Table 3 gives the average results for the two methods, the percentage error, and the percentage of ascaridole actually present in the samples.

TABLE 3.

Average percentage of ascaridole in samples found by the official and Paget methods.

	SAMPLE 1		SAMPLE 2		SAMPLE 3		SAMPLE 4	
	Ascaridole	Error	Ascaridole	Error	Ascaridole	Error	Ascaridole	Error
Official method	52.18	15.88	72.15	7.20	83.98	39.96	94.55	50.79
Paget method	44.37	1.40	66.76	1.09	60.44	.73	62.94	.09
Actually present	45.00		67.50		60.00		63.00	

NEW METHODS

Since a study of the methods for the determination of ascaridole was undertaken by the associate referee two new methods have been proposed for the estimation of the quality of oil of chenopodium. At the meeting of the association in 1929 attention was called to a colorimetric assay described by Knaffl-Lenz and Hofmann,[1] and recently Cocking and Hymas[2] have described a method based upon the liberation of iodine from potassium iodide by the organic peroxide ascaridole.

[1] *Arch. Pharm.*, **267**, 117 (1929).
[2] *Analyst*, **55**, 183 (1930).

Knaffl-Lenz and Hofmann devised an assay in which 1 cc. of concentrated hydrochloric acid is added to a 1 per cent solution of oil in alcohol, and the mixture is allowed to stand for a number of hours, six hours being considered sufficient. However, better results were obtained when the solutions were allowed to react for a period of 24 hours. The samples were then compared in a colorimeter with a standard solution of 100 per cent ascaridole treated in exactly the same way.

A few preliminary determinations were made upon seven oils with this assay. Oils B, C, and D were commercial samples whose ascaridole content had been checked repeatedly by the titanous trichloride method. Oils E, F, G, and H were samples of adulterated wormseed oil that were forwarded for collaborative assays and have been described.[1]

Three distinct assays were made upon each oil, the time of reaction being 6, 12 and 24 hours. It was found necessary to dilute each mixture with 5 cc. of alcohol in order to obtain a sufficient volume for use in a Dubosque colorimeter. Comparisons were made with a sample of highly purified ascaridole, as a standard, treated in the same manner as the samples. The results are given in Table 4, as are also the values of each oil by the titanous trichloride method.

TABLE 4.

Ascaridole found in samples (Knaffl-Lenz and Hofmann Colorimetric Method).

METHOD	TIME	B	C	D	E	F	G	H
	hours							
Colorimetric	6	67.80	73.50	68.20	54.00	62.00	57.40	61.00
"	12	68.60	56.00	62.70	45.50	67.70	56.70	61.00
"	24	68.60	59.20	63.60	44.50	65.70	60.4	55.80
Paget	—	74.30	60.00	65.40	44.5	67.00	60.20	62.80

The results obtained by this assay show considerable variation in the percentage of ascaridole found, the result depending on the length of time the samples were allowed to stand in contact with the hydrochloric acid reagent before the readings were made. Accurate readings were hard to obtain owing to the fact that dark insoluble oil droplets were present in every mixture that stood the prescribed time.

The presence of carbon tetrachloride and cineol as adulterants in samples E, F and G did not influence the results obtained. The authors of the method found that terpeniol alone, of a number of adulterants tested, gave a slight added color to the solutions. A solution of 50 per cent ascaridole in terpeniol gave a value of 55 per cent. Apparently this method deserves further consideration.

The iodine method proposed by Cocking and Hymas requires that 3 cc. of potassium iodide solution (83% W/V) in a stoppered tube of 60

[1] *This Journal*, 13, 334 (1930).

cc. capacity be mixed with 5 cc. of concentrated hydrochloric acid (31.81 per cent) and 10 cc. of glacial acetic acid and immediately cooled to −3°C. (limit 0° to −3°).

To this solution, after cooling, is added 5 cc. of the oil sample prepared by diluting 2.5 grams of oil to 50 cc. with 90 per cent acetic acid. The tube is then stoppered and allowed to stand in a cool place for 5 minutes. It may be left for 10 minutes if the temperature does not exceed 10°C. The contents are then titrated with 0.1 *N* thiosulfate. A blank is carried out precisely as described except that the final solution is diluted with 10 cc. of water before titrating. It was found necessary to employ an empirical factor in this method, the relation being 1 cc. of 0.1 *N* thiosulfate equals 0.00665 gram of ascaridole.

Determinations by this method were made upon the seven oils used in the colorimetric assay and, in addition, oil A, a sample of pure ascaridole, and oil J, a high-grade commercial oil.

Preliminary tests using the pure oil gave consistently low results. Variation of the amount of hydrochloric acid present and the time and temperature of the reaction showed no improvement. The amount of ascaridole present in the reaction mixture was changed by reducing the aliquots, and higher results were obtained. The results of these analyses on the 100 per cent ascaridole are shown in Table 5.

TABLE 5.

VOL OF ALIQUOT	ASCARIDOLE FOUND
cc.	*per cent*
5 (as prescribed)	89.43
3	93.73
2	96.80
1	101.43

As most of the oils analyzed contained not more than 75 per cent ascaridole, two assays were run on each sample, 5 cc. and 3 cc. aliquots, respectively, being used. The results appear in Table 6, together with the values obtained by the Paget method. One of the collaborators on the Paget assay voluntarily ran analyses upon the four samples, E, F, G and H, using the iodine method. These values are also contained in Table 6.

Although no conclusions can be drawn from such a preliminary trial of this assay there seem to be several objections to its adoption as a precise method. It has no advantages over the Paget method from the standpoint of time required to perform a determination, and it does not seem to be so accurate for high-grade oils. A freezing solution bath is an absolute necessity, for at room temperature both the liberated iodine and the concentrated hydrochloric acid present would decompose the ascaridole. Both the Paget and the iodine methods have an empirical factor

TABLE 6.

Ascaridole found in samples (Cocking-Hymas Iodide Method).

METHOD	VOL. OF SAMPLE	SAMPLE A	B	C	D	E	F	G	H
	cc.	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Iodine	5	89.43	72.43	56.63	61.67	45.05	62.63	65.60	59.13
Iodine	3	93.70	73.56	66.27	67.95	44.58	67.88	57.40	56.80
Iodine*						46.41	69.56	58.23	60.26
Paget		100.00	74.29	59.83	65.43	44.54	67.03	60.19	62.84

* Results reported by H J Fisher.

involved in the calculations. In the former method the factor is influenced only by a decided change in the hydrochloric acid concentration of the titanium trichloride solutions. In the iodine assay, according to the authors, there are probably three reactions occurring in sequence. There is probably a normal peroxide liberation of 2 moles of iodine from the acidified potassium iodide, followed by a further unexplainable liberation of iodine and then a reabsorption of iodine after its liberation, this reabsorption taking place when the solutions are diluted. Hence there are many chances for error in the results unless very rigid specifications are followed.

RECOMMENDATIONS[1]

Of the four methods studied, the titanous trichloride method proposed by Paget gives the most accurate estimate of the ascaridole content in oil of chenopodium. It is recommended that this method be made tentative and studied collaboratively next year.

Of the other methods studied, the Knaffl-Lenz and Hofmann colorimetric assay requires further study as to the length of time the samples should be in contact with the hydrochloric acid reagent.

Regarding the iodine method proposed by Cocking and Hymas, it has been pointed out that a number of factors influence the accuracy of the assay. It is recommended that the method be further studied.

The present Official U. S. P. method has been found inadequate as a measure of the percentage of ascaridole in oil of chenopodium. This is particularly true of oils adulterated with carbon tetrachloride, cineol, alcohol and cymene and the natural oils containing less than 90 per cent of ascaridole.

It is recommended that this assay be discontinued as the official method.

ACKNOWLEDGMENTS

The associate referee is highly appreciative of the interest taken in this work by the collaborators, and this opportunity is taken to thank W.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 53 (1931).

F. Reindollar of the Maryland State Department of Health, R. I. Grantham of Sharp and Dohme, H. J. Fisher of the Connecticut Station, C. Dalbom of the South Dakota State Laboratory, and G. S. Weiland of the University of Maryland, for their collaborative work on the samples sent out for study, and G. S. Weiland for his assistance during the study of the Knaffl-Lenz and Hofmann, and the Cocking and Hymas methods.

No report was made by the associate referee on salicylates and other phenols in mixtures because the subject was considered closed last year.

No report was given by the associate referee on small amounts of iodides in mixtures.

REPORT ON BISMUTH COMPOUNDS IN TABLETS

By J. CALLAWAY, Jr. (Food and Drug Administration, New York, N. Y.), *Associate Referee*

Work on this subject was begun last year, and a report was made[1]. In the method tried for determining bismuth in inorganic combinations by means of precipitating it as bismuth phosphate from an acid solution, the actual precipitation of the bismuth was found to be fairly satisfactory, but the method appeared to be applicable only in a restricted way. It was recommended that further work be done with the idea of developing a method which would be more generally applicable. Some time after the submission of the report of last year's work, the Contact Committee of the American Drug Manufacturers Association and American Pharmaceutical Manufacturers Association recommended a new method for the determination of bismuth compounds in tablets. This method should be applicable regardless of whether the bismuth compound is organic or inorganic, and it should also be applicable in the presence of the ordinary organic compounds used as excipients. It could not be used, however, in the presence of materials other than bismuth which would be precipitated by alkaline ammonium carbonate. This method provides for the destruction of the organic matter present by charring the material, treating with nitric acid, and then incinerating at a dull red heat.

It was decided to subject the Contact Committee's method to collaborative work this year and also to try the phosphate precipitation method of last year in connection with a wet oxidation of organic matter, using concentrated hydrogen peroxide in connection with nitric acid. Three samples were prepared. Sample A consisted of bismuth subgallate, starch and talc and theoretically contained 17.69 per cent of Bi_2O_3.

[1] *This Journal*, 13, 348 (1930).

Sample B contained bismuth betanaphthol, starch, lactose and talc with a a theoretical content of 25.69 per cent of Bi_2O_3. Sample C contained bismuth subsalicylate, starch, lactose and talc, with a theoretical content of 21.46 per cent of Bi_2O_3. Method 1 provided for wet oxidation with nitric acid and concentrated hydrogen peroxide and precipitation as bismuth phosphate. The details of the methods were as follows:

Method 1

Weigh 1.0 gram of the powdered and mixed sample into a 500 cc. Kjeldahl flask. Add 15 cc. of concentrated nitric acid, washing down any material adhering to sides of flask. Place on the steam bath or over a small flame and warm gently until the dense fumes of oxides of nitrogen first evolved are driven off. Now add cautiously 2–4 cc. of Superoxol. Allow reaction to subside. Heat on the steam bath or over a small flame for about 10 minutes. Add an additional 3 cc. of Superoxol. Continue heating and adding the oxidizing agent until a clear light-colored solution is obtained. (Small particles undissolved after several additions indicate talc and may be disregarded.)

Add about 50 cc. of water and boil for a few minutes to break up any excess peroxide. Transfer the solution to a 250 cc. beaker (filtering if appearance indicates any undissolved matter), washing flask and filter (if used) with 1 per cent nitric acid solution. The volume should be kept down as much as practicable. Next add 50 cc. of the phosphate reagent. A precipitate will be obtained, but it may be slow in forming. Adjust the acidity of the solution as follows: Add about 1 cc. of solution of thymol blue, then add slowly a saturated solution of ammonium acetate until the indicator changes color. Bring the contents of the beaker to boil and continue to boil gently a few minutes, then set on a hot plate or water bath for about an hour.

Collect the precipitate on a Gooch crucible, wash thoroughly with hot water, dry at 100°C., and ignite at dull red heat for 15 or 20 minutes. Cool and weigh as $BiPO_4$. Factor $BiPO_4$ to $Bi_2O_3 = 0.7664$. Calculate the percentage of Bi_2O_3 in the sample.

NOTE: The phosphate reagent is prepared by neutralizing 12 cc. of 85 per cent phosphoric acid with strong ammonia water and diluting to 1 liter.

Method 2 is the Contact Committee's method. The details are as follows:

Method 2

Weigh a quantity of tablets equivalent to at least 50 grains, but in no case less than 20 tablets. Place in a porcelain crucible of about 75 cc. capacity and heat slowly until the organic matter is thoroughly charred. Cool, add about 5 cc. of concentrated nitric acid, and again heat carefully until all the nitric oxides are driven off, then completely incinerate at dull red heat, keeping the temperature below the fusion point of Bi_2O_3. Cool, add an excess of nitric acid and digest on a steam bath to dissolve Bi_2O_3. When dissolved, cool, add 10–15 cc. of water and wash into a 100 cc. glass-stoppered graduated flask, using 1 per cent nitric acid for washing. Make up to exactly 100 cc. Filter, rejecting the first 10 cc. of filtrate.

Transfer a 10 cc. aliquot part of the solution to a 250 cc. beaker and add ammonia water in small portions, stirring after each addition until a slight permanent precipitate is obtained. Add ammonium carbonate T. S. in slight excess, with stirring, and heat on a steam bath for 1 hour. Cool and collect the precipitate in a tared Gooch crucible. Wash with water containing about 1 per cent ammonium carbonate and finally with a little hot water. Dry at 100°C., ignite, cool, and weigh as Bi_2O_3. Calculate the amount of bismuth oxide (Bi_2O_3) in the original tablets.

Reports were received from the following collaborators of the U. S. Food and Drug Administration:

	SAMPLE A—BISMUTH SUBGALLATE, STARCH AND TALC. THEORETICAL, 17.69% Bi_2O_3		SAMPLE B—BISMUTH B NAPTHOL, STARCH, LACTOSE AND TALC. THEORETICAL, 25.69% Bi_2O_3		SAMPLE C—BISMUTH, SUBSALICYLATE, STARCH, LACTOSE AND TALC THEORETICAL, 21.46% Bi_2O_3	
	(1)*	(2)†	(1)	(2)	(1)	(2)
Solomon Reznek	17.21	17.82	26.37	26.02	21.11	21.18
New York Station	17.19	17.80	26.12	26.00	21.76	20.98
T. N. Bennett	17.34	17.74	26.16	25.34	20.42	21.68
New York Station	17.34	17.90	24.83		20.34	21.46
			24.77	25.38		
			25.25			
Earl L. Anderson	17.69	17.48	25.12	24.53	21.11	21.07
Baltimore Station	17.83	17.67	25.46	24.58	21.17	21.43
E. O. Eaton	16.6	16.8	23.0	25.6	20.6	21.8
San Francisco Station	17.4	16.2	25.8	25.6	19.6	21.0

* (1) Phosphate method with wet oxidation.
† (2) Contact Committee method [$(NH_4)_2CO_3$ precipitation].

DISCUSSION

Both of these methods gave fairly good results on the three samples submitted for collaborative work. The Contact Committee method (2), however, appeared to be somewhat the better of the two. The associate referee had thought that Method 1 might be more generally applicable than Method 2 for bismuth in the presence of other compounds. This, however, is apparently not the case, as on a few trial determinations interference was noted in the presence of other metals except magnesium. The Contact Committee method is also applicable in the presence of magnesium compounds. Cerium compounds often accompany bismuth compounds in tablets. From a few experiments so far conducted, it would appear that neither method can be relied upon in the presence of cerium compounds.

Since this report was prepared, the following results of collaborative work on these samples were received from L. E. Warren, Food and Drug Administration, Washington, D. C.:

	SAMPLE A	SAMPLE B	SAMPLE C
	per cent	*per cent*	*per cent*
Method 1	16.61	24.12	21.35
	17.48	25.93	21.42
	17.41	22.43	21.81
Method 2	16.92	24.41	21.83
	16.79	24.46	21.68

He comments as follows:

I am not favorably impressed by Method I since I found it almost impossible to obtain satisfactory checks. I believe that the time allowed for the precipitation is not long enough and that the precipitation should be made in a solution of greater dilution. I found that in most cases if the directions were strictly followed the filtrate gave a further precipitation of bismuth phosphate on dilution with water and further warming.

RECOMMENDATIONS[1]

It is recommended that Method 2 be adopted as a tentative method for the determination of bismuth in tablets containing bismuth compounds with the proviso that this method is not applicable if the bismuth is accompanied by material that will be precipitated by alkaline ammonium carbonate.

REPORT ON COLORIMETRIC METHODS FOR VITAMINS

By H. J. FISHER (Agricultural Experiment Station, New Haven, Conn.), *Associate Referee*

No experimental work on colorimetric assay for vitamins has been carried out this year.

The only vitamin color reaction showing sufficient promise to be worthy of investigation is the antimony trichloride test of Carr and Price[2] for vitamin A, and most of the evidence for the value of this test is confined to its use in assaying cod liver oil.

As much work is now being done on this test in research laboratories, with contradictory results in many cases, it did not seem that anything of value could be gained by the necessarily limited experimental work of a few collaborators.

It should be mentioned in this connection that evidence is accumulating that vitamin A is not a chemical individual, the vitamin A from different sources reacting differently to various reagents. If this be true, it is not likely that, even if a satisfactory reagent for estimating vitamin A in one class of products were evolved, the same reagent could be applied to all classes of foodstuffs.

To the list of proposed colorimetric tests for vitamins in the bibliography of last year[3] a method recently proposed by Spruyt[4] for testing for vitamin B_1 should be added.

RECOMMENDATION[5]

It is recommended that collaborative work on colorimetric methods for vitamins be not undertaken until the tests have been more thoroughly investigated by the research laboratories now working on this problem.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 53 (1931).
[2] *Biochem. J.*, 20, 497 (1926).
[3] *This Journal*, 13, 352 (1930).
[4] *Chem. Weekblad*, 27, 298 (1930).
[5] For report of Subcommittee B and action of the association, see *This Journal*, 14, 53 (1931).

REPORT ON PHENOLSULFONATES

By E. H. GRANT (U. S. Food and Drug Administration, Baltimore, Md.), *Associate Referee*

The work of the associate referee on phenolsulfonates last year was concerned with methods depending on the bromination of these salts to tribromphenol, since it had been shown that the bromination to dibromphenolsulfonic acid by the U.S.P. IX method may not stop at the desired point.

This year the associate referee ran almost a hundred determinations in an effort to perfect one of these methods. Unfortunately the bromination does not stop with the formation of tribromphenol; bromine will continue to be absorbed so long as an excess is present. The highest degree of bromination obtained corresponded to about 5-2/3 atoms of bromine for each molecule of phenolsulfonic acid. The indications are that with the addition of more than three atoms of bromine the molecule is broken down completely. It is more difficult to stop the bromination at tribromphenol than at dibromphenolsulfonic acid.

PRELIMINARY STUDY

Studies made of the effect of various conditions and chemicals on the process of bromination showed that the controlling factors, in the order of their importance, were the amount of potassium bromide present, the amount of bromine in excess of theory, and the length of time allowed for reacting. It is possible under properly chosen conditions to run two parallel determinations under identical conditions as to time and concentration of reagents except as to the amount of potassium bromide used, and, by using different amounts of potassium bromide, get practically quantitative formation of the dibrom compound in one experiment and of tribromphenol in the other. Bromides slow down the formation of both compounds, but affect that of tribromphenol most. An excess of potassium bromide, therefore, favors the stopping of bromination at dibromphenolsulfonic acid.

In the liberation of bromine from a bromide-bromate solution, one molecule of $KBrO_3$ reacts with five molecules of KBr to yield six atoms of bromine; but in a reaction where the bromine is substituted for a hydrogen in an organic compound, three of these atoms of bromine enter into the organic radical and three are split off as hydrobromic acid and can then react with further quantities of bromate to yield bromine. It is necessary to use only five minus three, or two molecules of potassium bromide, to furnish enough for the complete reaction. There are then three ways to make up 0.1 *N* bromine:

(1) U.S.P. with a large excess of KBr—2.7835 gram of $KBrO_3$ and about 46.38 grams of KBr per liter after adjustment.

(2) Ratio 1:5, which immediately releases the total bromine—2.7835 grams of $KBrO_3$ and 9.9175 grams of KBr per liter.

(3) Ratio 1:2, which immediately releases 40 per cent of the bromine and releases the rest as substitution proceeds—2.7835 grams of $KBrO_3$ and 3.967 grams of KBr per liter.

The third solution is the only one where there would be no bromide present during the reaction to slow down the bromination. At any point in the reaction there would be 40 per cent as much free bromine present in using the third solution as there would be at the same point if either of the other solutions were used. All three solutions were tried out, and, as had been anticipated, the third one is the most effective for brominating to tribromphenol. There were also tried solutions of bromine in glacial acetic acid and in pure water, but these showed no signs of superiority and are not stable.

Some salts which do not enter into the reaction slow down the bromination and others hasten it. Mercuric chloride is an especially strong catalyst. A determination was run exactly according to the U.S.P. IX directions, except that 0.20 gram of sodium phenolsulfonate was used in place of 0.25 gram, and one gram of mercuric chloride in solution was added just before acidifying. This excess of bromine would ordinarily give a result of 101 per cent of theory based on the formation of dibromphenolsulfonic acid, but the presence of the mercury caused the results to jump to 132.67 per cent, or a 65 per cent conversion to tribromphenol. All of the salts tested affected the speed of bromination in one way or another. A reaction that is so sensitive to the catalytic action of foreign material is not reliable for use with mixtures of more or less unknown composition. No experiments were run to ascertain the effect of the different organic substances which might be encountered.

Hydrochloric acid was found to be the best means of liberating the bromine, with sulfuric acid (1+2) the next best. Weakly ionized acids will not cause any visible liberation of bromine, although there is a slow diminution of the titer of the reaction mixture, indicating that there is some bromination or oxidation going on.

RELIABILITY OF METHODS

Although the methods of determining phenolsulfonates by bromination do not seem reliable enough to be recommended for adoption, the results obtained were accurate enough to justify reporting them for the benefit of those interested in the analysis of such products.

The U.S.P. IX method is reliable to within about 1 per cent if catalysts are absent and the excess of bromine is kept between the equivalents of 2 cc. and 5 cc. of 0.1 *N*. This generally requires a preliminary examination, which can be carried through with a sufficient degree of accuracy by running the bromide-bromate solution from a buret into the acidified sample, and noting when the bromine ceases to be absorbed. It is ab-

sorbed very rapidly at first and then requires about 5 or 10 seconds to disappear. The end point is very faint but good enough for the purpose of determining approximately how much solution to use. Add about 3 cc. excess. Table 1 shows the results obtained by the U.S.P. method.

TABLE 1.
U.S.P. IX Method.
Zinc Phenolsulfonate, 1 cc. 0.1 N = 0.006947 gram

EXP. NO.	SAMPLE	0.1 N Br, U.S.P.	EXCESS	FOUND
	gram	*cc.*	*cc*	*per cent*
1	0.32	50	4.41	98.97
2	0.32	50	4.06	99.73
3	0.32	50	4.09	99.67
4	0.32	49	3.25	99.32
5	0.32	48	2.32	99.17
6	0.3328	50	2.32	99.53
7	0.32	47	1.39	99.02
8	0.32	50	4.29	99.23
9	0.32	50	3.83	100.23
10	0.32	50	4.27	99.28
	Sodium Phenolsulfonate, 1 cc. 0.1 N = 0.0058035 gram			
11	0.25	50	6.96	99.91
12	0.25	46	2.49	101.00
13	0.25	46	2.67	100.59
14	0.12	23	2.32	100.01
15	0.25	46	3.07	99.66
16	0.5	50	7.62	49.20
17	0.55	50	3.83	48.72

The zinc phenolsulfonate used was 99.88 per cent pure, and the sodium salt 100.03 per cent, as shown by determinations of the metallic element.

In experiments 4, 5, and 7, a preliminary titration was made, and after the addition of an excess of the bromide-bromate solution, the same mixture was used for the U.S.P. method. The results are a trifle low, but a better result was obtained in 6 where the sample was adjusted and the determination run strictly according to directions.

In experiment 8, two grams extra of KBr was added before acidifying. In experiment 9, enough cracked ice was added to reduce the initial temperature to about 16°C. before acidifying, and the ice may have introduced some impurities, such as ether fumes, to account for the slightly

high results. In experiment 10, the time of reaction was reduced to 5 minutes. Therefore, three out of four of the determinations made strictly according to the U.S.P. are within the experimental errors of an iodine titration. The results for sodium phenolsulfonate are not so good, however.

In experiments 14, 15, and 17, ice was added. Experiments 16 and 17 were run on the 1929 collaborative sample, a 50 per cent mixture of sodium phenolsulfonate with lactose prepared by the previous associate referee. The lactose may have an adverse effect on results.

To determine phenolsulfonates by bromination to tribromphenol, it is necessary to speed the reaction up as much as possible so as to reduce the time of action of the bromine and reduce the excess of bromine which must be added, and thereby reduce the amount of higher brominated products formed. To this end, the free hydrobromic acid must be eliminated and a catalyst added. Instead of the U.S.P. standard bromine, use the special 1:2 ratio mentioned earlier in this report. The directions call for just barely enough bromide over theory to allow for errors, etc. The method follows:

METHOD

So adjust the amount of sample as to allow about 5 cc. of 0.1 *N* Br in excess. Dissolve the sample in about 25 cc. of water in a glass-stoppered flask, add 50 cc. of special 0.1 *N* bromine (2.8 grams of $KBrO_3$ and 4.2 grams of KBr per liter, standardized as usual) and 20 cc. of 5 per cent $HgCl_2$, then add 5 cc. of concentrated HCl. Immediately stopper the flask and allow to stand, preferably in the dark, for 3 hours. Quickly add, in a manner to prevent escape of bromine, enough potassium iodide solution to redissolve the mercury. Titrate with 0.1 *N* $Na_2S_2O_3$, starch indicator, adding a few drops of chloroform near the last to dissolve the tribromphenol which may otherwise occlude iodine. 1 cc. of 0.1 *N* bromine = 0.004631 gram of zinc phenolsulfonate or 0.003869 gram of sodium phenolsulfonate.

Note that the factors in the two methods are different. Be careful to use the proper factor.

Table 2 shows the results by the tribromphenol method.

TABLE 2.
Tribromphenol method.
Zinc Phenolsulfonate

EXP NO.	SAMPLE	SPECIAL BROMINE	5% $HgCl_2$	CONC. HCl	TIME	EXCESS	FOUND
	gram	*cc.*	*cc.*	*cc.*	*hours*	*cc.*	*per cent*
18	0.2	64.51	none	5	2	21.58	99.40
19	0.256	60.30	5	10	2	5.63	98.90
20	0.256	60.30	5	5	3	6.99	98.25
21	0.256	60.30	20	8	3	5.16	99.75
22	0.256	60.30	none	8	3	5.21	99.61
			Sodium Phenolsulfonate				
23	0.175	50.25	20	8	3	4.75	100.59
24	0.175	50.25	20	8	3	4.15	101.37

Experiment 18 would suggest that, by using a greater excess of bromine the time could be shortened, but an excess of 12 cc. showed 95.64 per cent and an excess of 30 cc. showed 104.71 per cent, so it would be very difficult to adjust the excess properly. Experiment 22 indicates that the use of $HgCl_2$ may not be necessary. The action of mercury shows up very strikingly on the shorter periods, and probably is of advantage in the three-hour period. The results on sodium phenolsulfonate again run relatively higher.

The bromination of phenolsulfonates proceeds so slowly that it was not thought that a titration method using a dye indicator would be feasible, but a few experiments with the following method indicate that it is worthy of further study:

Dilute the sample to about 30 cc., add 5 cc. of concentrated HCl, and titrate with 0.1 *N* Br, U.S.P., with no indicator as long as the bromine is absorbed rapidly. When the bromine is absorbed slowly, wait about 10 seconds after the last addition and then add 1 drop of methyl orange indicator. Continue the titration, adding more indicator whenever the previous lot is practically completely bleached. At first only a few seconds need be allowed for absorption of bromine, but towards the last it is necessary to wait about 10 seconds before adding more indicator. The end point is reached when, after waiting 10 seconds and then adding a drop of indicator, the dye is slowly decolorized.

In this method, 1 cc. of 0.1 *N* Br = 0.006947 gram of zinc phenolsulfonate or 0.0058035 gram of sodium phenolsulfonate. Results obtained were 99.69 per cent zinc phenolsulfonate and 99.53, 99.82, and 99.53 per cent sodium phenolsulfonate, when methyl orange was used and 100.04 per cent zinc phenolsulfonate and 100.40 per cent sodium phenolsulfate when methyl red was used.

At ordinary temperatures the bromination of phenolsulfonates and also the bleaching of the dye are slow reactions, which makes the titration somewhat tedious. At higher temperatures the bromination goes beyond the formation of dibromphenolsulfonic acid. This method has the advantage that there is never any appreciable amount of free bromine present, so the danger of the formation of tribromphenol is small. The influence of catalysts ought to be negligible.

It is recommended[1] that titration methods using dyes as indicators be studied next year.

REPORT ON SULFONAL (SULFONMETHANE) AND TRIONAL (SULFONETHYLMETHANE)

By W. S. HUBBARD (Schwarz Laboratories, Inc., New York City), *Associate Referee*

In 1927 L. E. Warren[2] published a paper, entitled "Note on the Assay of Sulfonal Tablets," and in 1928[3] a similar paper regarding trional.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 53 (1931).
[2] *This Journal*, 10, 523 (1927).
[3] *Ibid.*, 11, 404 (1928).

Method I, proposed by Warren and since used by a number of manufacturers and State and Federal laboratories, was the method sent out to collaborators by the associate referee.

For the first year it seemed best to limit the work to trional and to the simplest method, which follows:

Method

Weigh approximately 0.5 gram of the powder, macerate in a small beaker with 10 cc. of chloroform, and decant the solvent through a small filter. Repeat the extraction with chloroform until the powder is exhausted of trional. Wash the filter with a few cc. of fresh chloroform and evaporate the united solvent in a weighed beaker at ordinary temperature (or at a temperature below 50°) by the aid of a gentle current of air, taking care near the end of the evaporation to rotate the container in an inclined position. Add 5 cc. of anhydrous ether to the residue and evaporate the solvent at ordinary temperature. Dry the residue to constant weight in a desiccator over sulfuric acid or calcium carbide.

The sample consisted of 75 per cent trional and 25 per cent starch. The following results were obtained:

COLLABORATOR	TRIONAL FOUND *per cent*	RECOVERY *per cent*
H. J. Fisher	73.44	97.92
Conn. Agr. Exp. Sta.	73.50	98.00
New Haven, Conn.		
Guy G. Frary	74.04	98.72
State Chemical Lab.	73.46	97.94
Vermilion, S.D.	74.70	99.60
Claude Dalbom	72.14	96.18
State Chemical Lab.	71.04	94.72
Vermilion, S.D.		
Wm. C. Cavett	73.54	98.05
Food and Drug Adm.	73.58	98.01
Chicago, Ill.		
F. L. Geiler	74.37	99.16
Univ. of West Virginia	73.54	98.05
Morgantown, W. Va.	73.26	
J. H. Loughbrey	73.8	97.68
Food and Drug Adm.	74.3	99.07
New York City		
C. Dayharsh	73.67	98.20
Schwarz Laboratories, Inc.		
New York City.		
L. E. Warren		
Food and Drug Adm.	73.5	98.00
Washington, D. C.	73.8	98.40

The trional used in the mixture sent to collaborators was furnished by Warren and was the same lot he had used in his work. It was found to conform to U.S.P.X. requirements.

After Dayharsh had obtained his results on this mixture he endeavored to find the cause of the low results. His comments are appended, together with some work on Method II, as so designated by Warren.

COMMENTS BY COLLABORATORS

G. G. Frary.—Mr. Dalbom used four portions of chloroform following the first 10 cc. called for by the method, making five portions in all. He used approximately 10 cc. each time.

I used on the first two trials six 10 cc. portions of chloroform, and on the third trial I used eight such portions.

It is my opinion that the method should specify the number of chloroform extractions to be made, or should provide some method for determining when the trional removal is complete. I do not know whether the fewer washings made by Mr. Dalbom account for his lower results, but I think there should be eight to ten washings with 10 cc. portions of chloroform.

C. Dayharsh.—In checking Method I for the assay of trional, I carefully prepared a mixture of 75 per cent trional and 25 per cent starch. The trional conformed to U.S.P.X. requirements. This I assayed several times and obtained from 73.2–73.8 per cent, the average being 73.6 per cent of trional recovered, as reported to the associate referee. This is also the same mixture which was sent to the other collaborators.

In like manner, I assayed a 75 per cent mixture of trional, U.S.P., with starch which had been thoroughly dried. In this case my average recovery was considerably lower than in the other case, being 72.2 per cent of trional. On this same mixture, I ran the assay, using ethyl ether in place of chloroform. The results obtained averaged 72.8 per cent of trional recovered. It would therefore appear that ether acts as a solvent slightly better than does chloroform.

It might be well to note here that drying in a desiccator for 24 hours is not quite sufficient to bring the residue to constant weight. More accurate results are obtained by drying from 36 to 48 hours in a desiccator. As for example:

AFTER 24 HOURS *per cent*	AFTER 48 HOURS *per cent*	DIFFERENCE *per cent*
98.46	98.15	0.31
99.14	98.89	0.25
73.81	73.17	0.64

In my opinion the step in Method I, "Macerate the powder in a small beaker with 10 cc. of chloroform and decant the solvent through a small filter," is unnecessary. Just as accurate results were obtained by transferring to a filter from a weighing bottle, a small portion of the sample, and determing the amount of sample taken by difference.

The recovery of trional by means of continuous extraction, according to Method II, using an alundum crucible in a Bailey extractor, has also been tried. Following the method exactly, except that the sample was mixed with 4 or 5 times its weight of ignited sea sand, and using chloroform, I find that my results are very low and vary greatly. For example, on a mixture containing 75 per cent of trional, my recovery was only 68.34 per cent in one case and 42.25 per cent in another case.

By substituting ethyl ether in place of chloroform, my percentage recovery,

though not satisfactory, was much better. The following amounts of trional were recovered: 72.94, 73.46, 74.04, and 76.34 per cent. Average, 74.36 per cent.

This again confirms my opinion that ethyl ether is a better solvent for trional than chloroform when using Method II. It may be that the higher temperature, needed to boil the chloroform, has partly volatilized the trional.

Another advantage of the ether over chloroform is the rapidity with which it evaporates in an air current.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for the determination of trional sent out this year be given further study, particular attention being given to the comments of Frary and Dayharsh.

(2) That the method using a Bailey or Soxhlet extractor be studied collaboratively and that both chloroform and anhydrous ether be used.

The associate referee desires to express his appreciation and thanks to L. E. Warren for his assistance and suggestions and to C. Dayharsh for his interest in preparing the samples and trying out Method II.

REPORT ON EMETINE

By F. C. SINTON (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Emetine hydrochloride is official in the U. S. Pharmacopeia, but no method of assay is provided therein, nor has this association recognized any method for the quantitative determination of emetine or any of its salts.

Emetine is the principal alkaloid of ipecac;[1] cephaeline and a minute amount of psychotrine are also present. Emetine [methyl-cephaeline ($C_{15}H_{22}NO_2$)] is an amorphous white alkaloid, readily soluble in alcohol, ether, or chloroform and sparingly in water. The melting point is 74°C.[2] It is used in medicine for its emetic, expectorant, and amoebicidal action.

According to the U.S.P. emetine hydrochloride contains variable amounts of water of crystallization and when dried to constant weight at 100°C. loses not more than 19 per cent (water). It is permitted to contain not more than 2 per cent cephaeline.

Cephaeline, due to its phenolic character, is not extracted by ether from a solution of fixed alkali, and the separation of emetine in the proposed method is based on this principle. Ether is specified as the solvent since cephaeline is readily soluble in chloroform.

For the work this year a product labeled "Emetine Hydrochloride U.S.P." was purchased on the market and found to comply with all the Pharmacopeial requirements. Analysis by titration, using methyl red indicator, showed 83.31 per cent of emetine hydrochloride anhydrous.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 53 (1931).
[2] Henry, Plant Alkaloids, 2nd ed., p. 420.

According to the U.S.P. tests for purity, 14.6 per cent water and 1.65 per cent cephaeline were present. This emetine hydrochloride was carefully mixed with 3 parts of milk sugar and accordingly contained 20.83 per cent of emetine hydrochloride anhydrous. Portions of the mixture, together with copies of the proposed method, were sent to the collaborators.

EMETINE IN TABLETS

REAGENTS

(a) *Sodium hydroxide solution.*—4 gram. to 100 cc.

(b) *Washed ether.*—Shake equal volumes of ether and water in a separatory funnel. Discard the water.

(c) *Neutral alcohol.*

(d) *Hydrochloric acid.*—0.02 *N*.

(e) *Methyl red indicator.*

PREPARATION OF SAMPLE

Weigh collectively all unbroken tablets and calculate the average weight per tablet. Powder a representative number of tablets, mix thoroughly, and place in a weighing bottle.

DETERMINATION

Transfer to a small separatory funnel sufficient of the powdered material, accurately weighed, to represent approximately 100 mg. of the alkaloidal salt. Dissolve in a minimum of water and add 5 cc. of the dilute sodium hydroxide solution. Extract with 30 cc. of washed ether, draw off the aqueous solution, and swirl the separatory funnel to remove water from sides. Wash the ether with 1 cc. of water, adding the wash water to the aqueous solution. Decant the ether into a third separatory funnel, washing the mouth of the separatory funnel with ether. Repeat the extrac-

RESULTS

ANALYST	PER CENT	AVERAGE
E. O. Eaton	20.2	20.1
San Francisco, Calif.	20.0	
E. M. Hoshall	21.34	21.43
Baltimore, Md.	21.57	
	21.44*	
	21.38*	
W. C. Cavett	20.86	20.93
Chicago, Ill.	21.0	
W. B. Kunke	19.8	19.7
Chicago, Ill.	19.6	20.89
F. C. Sinton	20.93	
Chicago, Ill.	20.85	
	Maximum	21.57
	Minimum	19.6
	Average	20.61

* Titrated by adding excess of acid and titrating back with 0.02 *N* KOH.

tions with 25, 20, 15 and 10 cc. portions of ether or until extraction is complete, washing with 1 cc. of water each time, and combine the ether extracts in the third separatory funnel. Filter into a beaker through cotton previously wet with ether, finally wash the separatory funnel with ether, and evaporate on a steam bath, using a low temperature to complete the evaporation.

To the residue add 2 cc. of neutral alcohol, cover the beaker with a watch-glass, and allow to reflux on the steam bath for a few minutes. Add a few drops of methyl red, and without dilution titrate with 0.02 *N* acid to a faint pink. Cover the beaker and digest on a steam bath until all particles are completely dissolved. Cool, and add about 30 cc. of recently boiled distilled water. Finish the titration with standard acid to a faint red.

1 cc. of 0.02 *N* acid = 5.6946 mg. of emetine hydrochloride ($C_{30}H_{44}O_4N_2 \cdot 2HCl$).

COMMENTS BY COLLABORATORS

E. O. Eaton: I should prefer to weigh the sample and dissolve in beaker and transfer to separatory funnel. Titration procedure is excellent.

E. M. Hoshall: The end point found, following the method submitted, is very uncertain, while in the modified method it is quite sharp. Although neutral alcohol was used in both cases, we have found in general that this is undesirable as the end point is not so sharp when alcohol is used.

W. C. Cavett: The method appears to be satisfactory.

W. F. Kunke: No trouble was encountered with the end point or in obtaining complete extraction.

DISCUSSION

The collaborators and associate referee found that the end point with methyl red indicator was satisfactory. However, Hoshall stated that the end point was uncertain, due to the presence of alcohol. It has been found in the titration of alkaloids that alcohol in quantity of 2 per cent does not interfere with the end point.

The average of the five results reported shows a variation of not more than 0.2 per cent from the theoretical. This is considered sufficiently accurate for adoption of the method.

RECOMMENDATION[1]

It is recommended that the method for the determination of emetine hydrochloride be made tentative.

REPORT ON CHLOROFORM AND CARBON TETRACHLORIDE

By JOHN R. MATCHETT (Prohibition Chemical Laboratory, Chicago, Ill.), *Associate Referee*

The problem for collaborative study this year was that of determining chloroform by distillation from mixtures containing non-volatile chlorides. Previous work by Moran[2] and by Willgerodt[3] has shown that with all methods of distillation used low results were obtained. The preparation of

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 53 (1931).
[2] *This Journal*, 10, 352, 358 (1927).
[3] *Am. J. Pharm.*, 97, 584 (1927).

this nature most frequently encountered is cough sirup. Samples for analyses were therefore prepared to simulate this product.

METHOD OF ANALYSIS

The method used for the determination of chloroform in the mixture is that of Roberts and Murray,[1] with a few suggestions by the associate-referee. The method and the suggestions follow:

REAGENTS

(a) *Alcoholic potassium hydroxide.*—Dissolve 30 grams of potassium hydroxide (free from chloride) in 30 cc. of water with sufficient methyl alcohol (reagent quality) to make 100 cc. Allow to stand 3 days, then draw from the top with a clean pipet (do not attempt to filter).

(b) *Alcohol.*—U.S.P. (95 per cent ethyl alcohol).

(c) *Nitric acid.*—U.S.P. (68 per cent nitric acid).

(d) *Silver nitrate.*—Dissolve 10 grams of U.S.P. silver nitrate in sufficient water to make 500 cc.

(e) *Phenolphthalein.*—Dissolve 0.1 gram of phenolphthalein in sufficient 95 per cent alcohol to make 100 cc.

DETERMINATION

Place 1 gram of calcium carbonate and 75 cc. of alcohol in a 250 cc. Kjeldahl distilling flask and carefully pipet into this mixture 20 cc. of the sirup to be examined, being careful not to agitate the mixture and to keep the tip of the pipet just below the surface of the liquid. Connect with a straight bore condenser and distil into a previously cooled citrate bottle immersed in cracked ice and containing 25 cc. of alcoholic potassium hydroxide solution into which the tip of the delivery tube extends.

When 70 cc. of the alcohol has been distilled over (this can be judged by previously marking the bottle) discontinue the distillation and wash the receiving tube with 10 or 15 cc. of distilled water, collecting the washings in the citrate bottle. Stopper the bottle and gently agitate, taking care to prevent the solution from coming in contact with the rubber washer.

Allow to stand overnight at room temperature, then heat on a steam bath for 1 hour. Remove from the bath and allow to cool, then empty the contents of the bottle into a 500 cc. beaker and wash the bottle with distilled water until the washings are no longer alkaline to phenolphthalein, adding each washing to the main solution. Now add 15 cc. of nitric acid and an excess of silver nitrate, stir well, and allow to stand in a dark place for 15 minutes.

Collect the precipitate upon a Gooch crucible which has been previously prepared, dried at 105°C. and weighed. Wash the precipitate with several portions of distilled water, then with 5 cc. of alcohol followed by a 5 cc. portion of ether. Dry at 105°C. and weigh.

Each gram of silver chloride corresponds to 0.027765 gram of chloroform. Assuming that 1 minim of water weighs 0.06161 gram and that the specific gravity of chloroform is 1.475 (average of the U.S.P. limits), then 1 minim of chloroform weighs 0.090936 gram, and basing calculations on 1 fluid ounce measuring 29.57 cc. the factor for grams of silver chloride to minims of chloroform per fluid ounce is 4.5142 for the 20 cc. sample.

[1] *Am. J. Pharm.*, **101**, 654 (1929).

NOTES BY ASSOCIATE REFEREE

1. There seems no good reason why the determination of chloride ions after saponification of the chloroform may not be made volumetrically.[1]

In order to carry out the determination, transfer the solution in the citrate bottle to a 200 cc. volumetric flask, rinsing the bottle until the wash water is no longer alkaline to phenolphthalein. Bring to room temperature, fill to the mark with water, and mix. Transfer a 50 cc. aliquot to a 100 cc. volumetric flask and acidify with nitric acid, adding about 2 cc. in excess. Add 25 cc. of 0.1 *N* silver nitrate solution, shake thoroughly, fill to the mark with water, and mix. Filter the mixture thru a dry filter into a dry flask, rejecting the first 20 cc. of the filtrate. To a 50 cc. aliquot of the filtrate, add 3 cc. of ferric ammonium sulfate indicator and titrate the excess silver nitrate, using 0.05 *N* ammonium or potassium thiocyanate.

The aliquots indicated should be satisfactory for the amount of chloroform contained in the average sample of cough sirup. If the amounts were greater or less, it would probably be more satisfactory to use smaller aliquots in case the amount of chloroform were greater, or a larger sample in case the amount were less.

For the determination by this method, it would of course be necessary to add standard 0.1 *N* silver nitrate solution and standard 0.05 *N* ammonium or potassium thiocyanate solution to the list of reagents given above.

2. Because of the small solubility of chloroform, it is necessary that the sirup be thoroughly shaken before the sample is removed. In samples containing small amounts of chloroform, an appreciable amount may be lost in the space above the sirup if the bottle is not kept well filled.

3. It has been observed that a saturated solution of potassium hydroxide in methyl alcohol saponifies chloroform more rapidly than the solution given, which contains about 30 per cent of water.[2] It is therefore suggested that a solution which contains 35 grams of potassium hydroxide per 100 cc. may be used to better advantage than the solution given in the list of reagents.

4. It is suggested that results be reported in grams of chloroform per 100 cc. This seems to the associate referee a more logical procedure than that of reporting minims per fluid ounce in spite of the fact that chloroform content is commonly labeled after the latter fashion. Each cubic centimeter of 0.1 *N* silver nitrate solution consumed = 0.00398 gram of chloroform.

5. It is necessary to place the bottle in a water bath at room temperature and gradually heat to boiling. The bottle should, of course, be covered with a towel or otherwise protected in order to prevent injury in case it should burst.

[1] Kunke, *This Journal*, 12, 50 (1929).
[2] *Ibid.*, 264.

PRELIMINARY WORK BY THE ASSOCIATE REFEREE

A sample of known chloroform content was prepared in the following manner:

Sixty-seven and five-tenths cc. of alcohol was placed in an Erlenmeyer flask and to this was added 17.5 cc. of a sirup containing approximately 50 grams of sugar and 4 grams of ammonium chloride per 100 cc.

Ten cc. of a solution of chloroform in alcohol containing 1.9715 grams of chloroform per 100 cc. was added to this mixture. The chloroform contained in the sample is therefore 0.19715 gram.

This mixture was then distilled (without the use of calcium carbonate), and the chloroform was determined by the volumetric modification of the above method. Three identical samples were thus analyzed. The results are given below:

CHLOROFORM CONTENT *gram*	CHLOROFORM FOUND *gram*	RECOVERY *per cent*
0.19715	0.1933	98.1
0.19715	0.1926	97.8
0.19715	0.1954	99.2

PREPARATION OF COLLABORATIVE SAMPLE

One liter of a sirup containing 436 grams of sugar and 43.6 grams of ammonium chloride was first prepared. The specific gravity was determined and found to be 1.162; 110 cc. of this solution was weighed into a 4-ounce bottle together with an additional 0.58 gram.

A 100 cc. measuring flask containing about 30 cc. of alcohol was weighed accurately. About 7.5 cc. of chloroform was added. The flask was quickly stoppered and weighed again. The weight of chloroform was found to be 11.3297 grams. The flask was then filled to the mark with alcohol and carefully mixed.

Ten cc. of this chloroform solution was then added to each bottle to prepare exactly 120 cc. of a preparation simulating cough sirup. Shrinkage, on addition of the alcohol, was allowed for by the addition of the 0.58 gram of sirup mentioned above.

The weight of chloroform thus introduced into each bottle was 1.3297 grams, which is equivalent to 0.9441 gram per 100 cc. In addition to the chloroform, the final sample contained approximately 40 grams of sugar and 4 grams of ammonium chloride per 100 cc. and approximately 8 per cent of alcohol by volume.

Eight identical samples were thus prepared on June 12, 1930, and four of them were analyzed during August and the first half of September, 1930. The results obtained are shown in the table, page 364.

COMMENTS BY COLLABORATORS

C. K. Glycart.—It was noted that on the addition of 1 gram of calcium carbonate as directed violent bumping occurred during distillation in the first determination.

When the quantity of the calcium carbonate was reduced to 0.1 gram. no bumping resulted. It appears that the addition of alcohol prolongs the time of distillation, which tends to facilitate the absorption of the chloroform in the alcoholic potash solutions.

W. F. Kunke.—Powdered calcium carbonate was used in the first determination, and since considerable bumping occurred, small pieces of soft marble were used instead of the powdered calcium carbonate in the other determinations. No more trouble was had with bumping.

Results obtained by collaborators.

SAMPLE NO.	METHOD	ANALYST	CHLOROFORM FOUND *gram per 100 cc.*
1	Gravimetric	Dalbom*	0.64
			0.65
2	Gravimetric	Glycart	0.61
			0.60
3	Gravimetric	Kunke	0.52
			0.50
			0.45†
	Gravimetric	Glycart	0.53
4	Volumetric	Matchett	0.61
			0.64
	Gravimetric	Kunke	0.52‡
	Volumetric	Shaffer	0.51§
			0.53§

* Analysis made in South Dakota State Laboratory under direction of G. G. Frary, State Chemist.
† Only 60 cc was distilled.
‡ Two samples had been removed from bottle two days previous to taking of sample. The receiving flask was not chilled, and the distillate did not stand overnight before heating.
§ Two samples had been removed from the bottle by Matchett two days previous to the taking of this sample and one more had been removed by Kunke the same day.

In Determination No. 3 (see collaborative results) only 60 cc. instead of 70 cc. as the method directs, was inadvertently distilled. No doubt the low result is due to this mistake and indicates that the chloroform distils very gradually.

In Determination No. 4 the distillate was not chilled with ice and was not allowed to stand overnight before heating. This determination was an experiment to learn if lower results would be obtained. Chilling very markedly retards the reaction. (Compare Experiments 1 and 3 with Experiment 22 in a previous[1] report.)

The alcoholic potassium hydroxide reagent is the same as I used and designated Reagent A in my report. Since this reagent is considerably diluted by the 70 cc. distillate, it would seem advisable to employ the more active alcoholic potassium hydroxide reagent (35 grams of potassium hydroxide in sufficient methyl alcohol to make 100 cc.) and designated by me as Reagent B. (Compare Experiment 1 with No. 2, same reference.)

J. R. Matchett.—When one gram of powdered calcium carbonate was used in the method as directed, violent bumping occurred during distillation.

[1] *This Journal*, 12, 267 (1929).

All determinations reported by me were made without the use of calcium carbonate and in these cases distillation proceeded smoothly without any bumping provided the flame was kept sufficiently low. The distillation should proceed so slowly that the liquid in the distilling flask scarcely appears to boil. About one-half hour is required.

All the samples analyzed by me were distilled in a 250 cc. Erlenmeyer instead of a Kjeldahl flask as directed, and a coil condenser was used with a long adapter reaching below the surface of the alcoholic potassium hydroxide solution.

A saturated solution of potassium hydroxide in methyl alcohol, containing about 35 grams per 100 cc., was used by me instead of the reagent directed in the method containing about 30 per cent water.

DISCUSSION OF RESULTS

It is apparent from the results given that a considerable amount of chloroform was lost from the samples between the time of preparation and that of analysis. The bottles were kept tightly stoppered during this time, and it is believed that chloroform would escape from any bottle unless very great precaution were taken.

The sample designated as No. 4 was originally opened by Matchett, and two samples were removed. Analyses of these samples gave 0.61 and 0.64 gram of chloroform per 100 cc., respectively. After being allowed to stand two days the bottle was again opened by Shaffer, and two samples were removed; analysis showed them to contain 0.51 and 0.53 gram of chloroform per 100 cc., respectively. These results indicate the loss of approximately 0.1 of a gram per 100 cc. on standing two days, during which time the bottle was not entirely full, thus offering greater opportunity for the chloroform to evaporate. It is believed from the large loss on only two days' standing, during which time conditions were favorable for evaporation, that this loss is actually due to evaporation and not to decomposition of the chloroform.

The bottle was opened, and the sample was removed by Kunke the same day that Shaffer's analysis was made. Analysis showed 0.52 gram of chloroform per 100 cc., which corroborates Shaffer's results. However, some variations were introduced into the method and the result is to be considered as confirmatory, rather than conclusive in this case. (See ‡ in the table.)

The results obtained by the associate referee on samples into which a positively known amount of chloroform was introduced indicate that the discrepancy between the amounts of chloroform introduced into the collaborative samples and the results obtained are due in fact to loss of chloroform on standing and are not indicative of a faulty method.

Particular attention is called to the fact that when only 60 cc. was distilled, low results were obtained († in table). Evidently the chloroform distils very gradually, and it is necessary to distil the full 70 cc. if good results are to be obtained.

DISCUSSION OF METHOD

It is recommended that the use of powdered calcium carbonate in the distillation flask be eliminated or reduced to 0.1 gram or that it be employed in granular form.

The use of the stronger alcoholic potassium hydroxide solution is suggested. The results, however, indicate that either solution is satisfactory.

The use of an Erlenmeyer flask instead of a Kjeldahl flask for the distillation gives equally satisfactory results, and in the opinion of the associate referee is more convenient.

RESULTS ON CARBON TETRACHLORIDE

Nine samples of carbon tetrachloride were analyzed by Lloyd E. Dale, who used the same method and obtained the results given below. These samples were prepared in exactly the same manner as those given in the preliminary work on chloroform. The first five were run as preliminary samples without distillation. The others were distilled before saponification as indicated.

SAMPLE NUMBER	CCl_4 CONTENT *gram*	CCl_4 FOUND *gram*	PERIOD OF HEATING *hours*	RECOVERY *per cent*
1	0.2537	—*	None	0.00
2	0.2537	0.2166	1	85.4
3	0.2537	0.2215	1	87.3
4	0.2537	0.2449	2	96.5
5	0.2537	0.2468	2	97.3
6	0.2726	0.2550†	2½	93.5
7	0.2726	0.2618†	3	96.0
8	0.2726	0.2640†	3	96.8
9	0.2726	0.2618‡	3	96.0

* Stood overnight at room temperature.
† Distilled with 80 per cent alcohol
‡ Distilled with 95 per cent alcohol—80 cc was distilled.

DISCUSSION OF RESULTS

The first five samples were analyzed without distillation. These stood overnight and were then heated in a boiling water bath for various periods of time as indicated in the table. Sample No. 1 was not heated at all. On adding silver nitrate after acidifying with nitric acid, the solution became turbid but no precipitate formed.

It is evident that standing overnight is not of any value in the case of carbon tetrachloride. The low results obtained by heating only one hour in the water bath indicate that a greater length of time is required for complete saponification.

Samples 6, 7, 8, and 9, were distilled as indicated. Because the boiling points of carbon tetrachloride and alcohol lie so close together (76° and 78°) it is necessary either to distil more than 70 cc. or to dilute the alcohol used in the distillation in order that the boiling point of the solution

may rise high enough to insure complete distillation of the carbon tetrachloride.

It spite of these precautions, it is evident from a comparison of the results obtained with distillation and those obtained without, that some carbon tetrachloride is lost in the distillation.

Sample No. 6 was heated in a water bath for two and one-half hours, and Samples No. 7, 8 and 9 were each heated three hours. The low results obtained on Sample No. 6 indicate that at least three hours' heating is necessary for complete saponification.

It is the opinion of the associate referee that this method is not sufficiently accurate to warrant adoption. Since, however, the use of carbon tetrachloride mixtures is rather uncommon in the United States and since the available methods applicable jointly to chloroform and carbon tetrachloride are sufficient for practically all cases, further work on carbon tetrachloride mixtures hardly seems necessary.

RECOMMENDATION[1]

It is recommended that the method for the determination of chloroform in mixtures, described above and studied by the associate referee and his collaborators during the present year be adopted as a tentative method with final adoption as official in view.

REPORT ON GUAIACOL

By N. L. KNIGHT (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

Apparently there is no published method specifically devised for the quantitative determination of guaiacol. U. S. Pharmacopeia X, p. 185, gives qualitative chemical and physical tests for identity and purity of the compound, but no method of assay. Chernoff[2] has published a gravimetric method for the determination of guaiacol carbonate which is based on a method for phenol quoted by Autenreith[3]; the method probably could be adapted to the determination of guaiacol itself, but since it requires at least two hours for one determination and was not applied to quantities of guaiacol carbonate of less than 0.1 gram, it seemed advisable to investigate other methods first. Chernoff also quotes a method for the direct volumetric measurement of guaiacol liberated from guaiacol carbonate, as described by Fernau[4], but this is obviously applicable only to relatively large quantities of the substance.

There have been found in the literature a few references to qualitative tests for a number of related phenols and phenolic compounds, among which guaiacol is sometimes mentioned. It is possible that some of these

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 53 (1931).
[2] *J. Am. Chem Soc*, 51, 3072 (1929).
[3] Detection of Poisons and Powerful Drugs, 5th Am ed., p. 31.
[4] *Z. Oesterr. Apoth. Ver.*, 49, 165 (1911).

tests might be adapted to quantitative colorimetric work. Ware[1] describes color tests for phloroglucinol and resorcinol, using hydrogen peroxide either alone or with dihydroxy-acetone or formaldehyde. Weinland and Binder[2] note a color reaction between catechol and ferric chloride in alkaline solution. Maue[3] obtains a color reaction with an alcoholic solution of guaiacol, ferric chloride, formaldehyde, and concentrated sulfuric acid. The possibilities of these tests have not been investigated.

Some preliminary work has been done with a colorimetric method, originally applied to monohydric phenols by Henningsen,[4] using the phosphotungstic-phosophomolybdic phenol reagent of Folin and Denis.[5] Henningsen uses beta-naphthol as his colorimetric standard and applies the method to meta-cresol, thymol, isoamyl phenol, ethyl phenol and butyl phenol. Using the same standard the method was applied to a solution of guaiacol in very dilute sodium hydroxide (25 cc. of 25 per cent NaOH per liter of solution). The guaiacol was present in a concentration of 0.0011 gram per 100 cc. Erratic results were obtained owing to the fact that the Folin-Denis reagent does not give the same tint with beta-naphthol as with guaiacol. Resorcinol and phloroglucinol were successively substituted for beta-naphthol as standards, and although they produced colors that could be matched perfectly with that of guaiacol, their color-intensity was greater than that required by the findings of Henningsen, who states that the color intensity is proportional to the molecular weight of the substance. The results with resorcinol indicate that its color is approximately three times as intense as it should be by this rule. Correcting by this factor, the results with resorcinol are much better than with beta-napthol, and it is thought that by taking the average of an extended series of readings an empirical factor may be obtained that is sufficiently accurate for all practical purposes.

The guaiacol used in this and in subsequent experiments satisfies the U.S.P.X tests for purity, and the resorcinol had a m.p. of 105°C., as against 110°C. for the pure substance according to Van Nostrand's manual.

Another possible method is suggested by the procedure for the determination of thymol developed by F. L. Hart.[6] Starting with the dilute alkaline solution of guaiacol aforementioned, to which hot distilled water and concentrated hydrochloric acid had been added, attempts were made to titrate with sodium bromate solution, using the permanent fading of methyl orange as the end point. A deep, turbid, chocolate-brown color at once developed, apparently due to the formation of a colored compound, as it persisted after days of standing and at last partially precipitated.

[1] *Quart. J. Pharm. Pharmacol*, 2, 267 (1929).
[2] *Ber.*, 45, 148 (1912).
[3] *Pharm. Ztg.*, 62, 255 (1918); *J. Soc. Chem. Ind.*, 37, 485A.
[4] *Ind. Eng. Chem.*, 15, 406 (1923).
[5] *J. Biol. Chem.*, 22, 305 (1916).
[6] *This Journal*, 12, 55 (1929).

Assuming that di-brom-guaiacol was formed, the next procedure attempted was to add an amount of sodium bromate solution in excess of the calculated amount necessary, allow to brominate on the steam bath, and then determine the excess bromine by adding potassium iodide and titrating the liberated iodine with sodium thiosulfate (starch indicator). It was quickly discovered that by careful adjustment of the added amounts of hot water, concentrated hydrochloric acid, potassium iodide, and time on the steam bath, and by adding only a slight excess of sodium bromate, an accuracy of the order of plus or minus 0.5 per cent could be obtained. But this involved previous knowledge of the amount of guaiacol present and therefore could not be applied to an unknown.

On adding large excesses of sodium bromate the results indicated that even more highly brominated guaiacols than the di-brom compound were formed. Also, the large volumes of free bromine produced persistently blew out the glass stoppers of the flasks on the steam bath and escaped, thereby invalidating the determinations.

It is thought that by carrying on the bromination at room temperature or in a pressure flask on the steam bath the method may be made practicable. Also, the sodium bromate titration may be adapted to guaiacol by the use of some indicator other than methyl orange and which, like it, contains an azo-group; it must also give a color which will be perceptible in spite of the brown coloration present. These specifications suggest methyl violet, methyl green, and benzopurpurin.

Four principal lines of future research are therefore indicated: Trials of new indicators in the bromate titration method; modification of the thiosulfate titration method; additional work on the colorimetric method using the Denis-Folin reagent; and trials of the gravimetric method of Chernoff.

None of the methods discussed have as yet been sufficiently developed to warrant the submission of samples to collaborators.

It is recommended[1] that this study be continued.

REPORT ON CALCIUM LACTATE

By ERNEST C. DEAL (U. S. Food and Drug Administration, New Orleans, La.), *Associate Referee*

The work undertaken was a collaborative study of an application of the ether extraction method in the determination of calcium lactate. It was hoped that the method, if proved satisfactory, might be applied to the analysis of other substances containing lactic acid, free or in combination. Several years ago essentially the same method[2] was proposed for determining lactic acid in tomato catsup. With the improved Palkin

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 54 (1931).
[2] Bacon and Dunbar Method—Leach, 4th ed.

extractor it was thought that better results might be obtained and that the method might prove of value.

Samples of two solutions were distributed. Solution No. 1 contained calcium lactate and water. Solution No. 2 was the same solution with the addition of 0.25 per cent calcium chloride.

Reports on the results obtained by the permanganate and oxalic acid method were received from five collaborators. These results, not at all concordant, are listed below:

COLLABORATOR	LACTIC ACID FOUND		PERCENTAGE OF RECOVERY	
	No. 1	No. 2	No. 1	No. 2
	gram	*gram*		
A.H.A.	0.133	0.146	91.1	100
J.A.B.		0.129		88.4
J.H.L.	0.093		63.7	
H.H.M.	0.118	0.114	80.8	78.1
I.S.S.	0.028	0.028	19.2	19.2

From the above results it is evident that the method is not reliable. Complete extraction is difficult if not impossible.

RECOMMENDATIONS[1]

It is recommended—

(1) That consideration of lactic acid in drug products be discontinued.

(2) That the present U.S.P. method be adopted for the assay of calcium lactate since calcium is the active constituent.

REPORT ON IODOFORM

By WM. F. KUNKE[2] (Food and Drug Administration, Chicago, Ill.), *Associate Referee*

Iodoform is a new subject so far as consideration by the Association of Official Agricultural Chemists is concerned. U.S.P.X includes iodoform, but no assay is given, and a review of the literature revealed no accurate and satisfactory quantitative method for the determination of iodoform. Schmidt's[3] original method and modifications were tried out, but the results were not satisfactory. The details of the procedure, modifications, and quantitative results are given later in this report. The present A.O.A.C. tentative method for the determination of chloroform[4] appeared to be worthy of trial. Unsatisfactory results, 96.7 per cent and 97.6 per cent, were obtained, although the more active alcoholic potassium hydroxide, Reagent (b), and considerably longer reaction periods were used. Leslie Hart[5] reported that he obtained 80.9 per cent by using the A.O.A.C.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 54 (1931).
[2] Presented by L. E. Warren.
[3] Pharmazeutische Chemie, 1910, Vol. 2, Organische Chemie, Part 1, p. 182.
[4] *This Journal*, 12, 50 (1929).
[5] Private communication.

method for chloroform, which specifies alcoholic potassium hydroxide, Reagent **(a)**. The writer,[1] when Associate Referee on Chloroform and Carbon Tetrachloride, found that the alcoholic potassium hydroxide, Reagent **(b)** (35 grams of potassium hydroxide dissolved in sufficient methyl alcohol to make 100 cc.) is a more active saponification reagent than the alcoholic potassium hydroxide, Reagent **(a)** (30 grams of potassium hydroxide dissolved in 30 cc. of water and diluted with methyl alcohol to make 100 cc.). In four experiments using 0.1313 gram of iodoform and 25 cc. of alcoholic potassium hydroxide, Reagent **(b)**, in each case and left to stand at room temperature for 2, 3, 4, and 5 hours the results obtained were 29.0, 39.0, 36.3 and 32.4 per cent, respectively. Under the same conditions, chloroform was almost quantitatively saponified (about 97 per cent) in one hour.[2] The treatment of iodoform with alcoholic potassium hydroxide by heating under a reflux was not tried because experiments showed that iodoform is considerably less readily saponified than is chloroform and the writer found that chloroform could not be accurately determined by such treatment. The Carius method was not tried because it was thought advisable to develop a simpler and equally accurate procedure.

After considerable experimental study a simple, rapid and accurate method was devised. The procedure is based upon the quantitative study of the reaction of iodoform with silver nitrate under different conditions but without the use of applied heat and a closed container. It was found that when an alcoholic solution of iodoform is treated with an aqueous 0.1 *N* silver nitrate solution and nitric acid under certain conditions the iodine of the iodoform will combine quantitatively with the silver. The excess silver, which should be approximately equal to the quantity of silver consumed by the iodine of the iodoform, may be titrated or the silver iodide formed may be weighed and the iodoform calculated.

The method was subjected to collaborative study. The results obtained by the collaborators are included in this report. No work on iodoform in mixtures was done.

IODOFORM SAMPLE USED

A sample of iodoform labeled U.S.P., prepared by a reputable manufacturer and bought in the open market, was tested by the U.S.P.X tests. It yielded no ash or water-soluble yellow coloring matter and complied with all the other requirements of the U.S.P.X, which allows a maximum tolerance for moisture of 1 per cent.

A 1-gram sample of iodoform weighed in a tared beaker was placed in a desiccator over sulfuric acid, and the loss in weight in 60 hours was 0.15 per cent. As the atmosphere in the desiccator contained free iodine it was assumed that the loss in weight was due in part to the decomposi-

[1] *This Journal*, **12**, 270 (1929).
[2] *Ibid.*, 267.

tion of the iodoform and in part to moisture. The sample contained no free iodine and water-soluble halide. Obviously, there was no necessity for purification of the iodoform. All the results given in this report, which are expressed in percentage, are based on the quantities of iodoform weighed out for samples, no allowance being made for the maximum possible moisture content of 0.15 per cent.

VOLATILITY OF IODOFORM

U.S.P.X states that iodoform is slightly volatile even at ordinary temperatures and distils slowly with the vapor of water. In connection with the weighing of iodoform samples it was desired to learn more accurately what the rates of volatility are under various conditions. A few experiments were made. In each case a gram of iodoform was weighed in an open, tared, 50 cc. beaker and placed under conditions likely to be used in a quantitative method, as shown below.

	LENGTH OF TIME *hours*	LOSS IN WEIGHT *per cent*
Exposed to air at room temperature	1	Not weighable
Exposed to air at room temperature	60	0.30
In desiccator over sulfuric acid	60	0.15
Heated at about 80°C.	½	7.0

It was learned from these experiments—

(1) That the rate of volatility (0.005 per cent by weight per hour) is so slow that it is negligible.

(2) That iodoform over sulfuric acid slowly decomposes with the liberation of iodoform.

(3) That iodoform is appreciably volatile at 80°C.

PRELIMINARY STUDY OF REACTION OF IODOFORM WITH SILVER NITRATE

Schmidt[1] gives a method which, briefly stated, consists of carefully heating on a water bath an ether solution of iodoform with an excess of 0.1 *N* silver nitrate and a few drops of nitric acid until no odor of ether or nitric acid remains. After cooling, 100 cc. of water is added: the excess 0.1 *N* silver nitrate is titrated with 0.1 *N* ammonium sulfocyanate, ferric ammonium sulfate being used as indicator, and the iodoform is calculated from the quantity of 0.1 *N* silver nitrate consumed. Three experiments were made, the first with the Schmidt method and the other two with a modified Schmidt method. In each experiment 20 cc. of 0.1 *N* silver nitrate solution was added to the iodoform (0.1313 gram) dissolved in 25 cc. of ether contained in a 200 cc. Erlenmeyer flask. The result of 91.6 per cent was obtained in Experiment No. 1.

In Experiments Nos. 2 and 3 the procedure was modified by inserting a funnel in the neck of the flask and after the ether was nearly evaporated,

[1] *Loc. cit.*

two additional portions of 20 cc. of ether each were added in each case. Also, in place of the few drops of nitric acid (68 per cent) specified in the method, 5 drops and 3 cc. were used respectively. The total time required for the heating was about 1 hour. The results obtained were 93.0 per cent

TABLE 1.

Preliminary quantitative experiments

(Results obtained by Associate Referee.)

EXPERIMENT NO.	ALCOHOL	0.1 *N* SILVER NITRATE (Aqueous)	NITRIC ACID (68%)	REACTION PERIOD	ALTERNATIVE METHOD	IODOFORM FOUND
	cc.	*cc.*	*cc.*	*hours*		*% by weight*
1	25	10	—	1	Vol.	37.8
2	10 plus 20 cc. ether	20	—	1	"	67.2
3	—	20	5	3	"	36.3
4	—	20	5	18	—	—*
5	—	20	5	384	Vol.	100.0
6	—	20	10	1	"	81.2†
7	20	10	1	2	"	87.8
8	20	20	1	2	Grav.	98.1
9	20	20	1	2	"	94.9
10	20	20	1	18	"	99.4
11	30	10	5	17	"	93.0
12	60	20	10	2	"	93.5
13	60	20	10	2	"	94.0
14	20	20	5	1	Vol.	95.2‡
15	20	20	5	1½	"	96.7‡
16	20	20	5	1½	"	97.2‡
17	20	20	5	1½	"	96.7‡

* Iodoform still remaining after 18 hours.

† The 10 cc. of nitric acid was added direct to the iodoform, then allowed to stand 20 minutes, before the 0.1 *N* silver nitrate was added

‡ The iodoform was dissolved in the alcohol and allowed to stand overnight before the 0.1 *N* silver nitrate and nitric acid were added.

and 98.0 per cent, respectively. These modifications appear to improve the method. However, when heat is used in the determination of iodoform, it is believed that inaccurate results are likely to be obtained due to the loss of iodoform by volatilization.

According to the literature, alkyl halogen compounds do not react readily with silver nitrate in aqueous solution. Greshoff[1] reported that iodoform reacts with a 20 per cent silver nitrate in aqueous solution, but no results were given to show how far the reaction proceeds. When alkyl halogen compounds are heated with silver nitrate in alcoholic solution, they undergo double decomposition and give esters.

An experimental study of the reaction of iodoform with silver nitrate

[1] *Ibid.*, p. 181.

under varying conditions was made. The reaction no doubt proceeds according to the following equation:

$$CHI_3 + 3\,AgNO_3 + H_2O = 3\,AgI + 3\,HNO_3 + CO$$

Table 1 gives the different variables and the quantitative results obtained. In each experiment the sample of iodoform (0.1313 gram) dissolved in the given volume of alcohol contained in an Erlenmeyer flask was treated with the given quantities of aqueous 0.1 *N* silver nitrate solution and nitric acid, and the reaction mixture was left to stand at room temperature for the time indicated. The silver consumed was determined by the Volhard method or the silver iodide formed was collected on a weighed Gooch crucible, washed, dried, and weighed. One cc. of 0.1 *N* silver nitrate solution = 0.01313 gram, or 1 gram of silver iodide = 0.5590 gram of iodoform.

DISCUSSION OF RESULTS (TABLE 1)

Iodoform does not readily react with aqueous 0.1 *N* silver nitrate (Experiments 3 and 4, Table 1) even though 10 cc. of nitric acid (68 per cent) and 20 cc. of 0.1 N silver nitrate are used for a 0.1313 gram sample of iodoform (Experiment 6, Table 1). However, the iodoform will react quantitatively with aqueous silver nitrate, but only after a very long reaction period (Experiment 5, Table 1).

Iodoform in alcoholic solution and aqueous silver nitrate do not readily react without the aid of nitric acid (Experiments 1 and 2, Table 1).

Considerable excess of silver nitrate is necessary. (Compare Experiments 7 and 8, Table 1.) This might be expected because when ionic silver is removed from the system by formation of silver iodide, as the reaction proceeds the unconsumed silver is present in progressively lower concentration. In the proposed method it was decided to specify the use of approximately twice the volume of aqueous 0.1 *N* silver nitrate solution theoretically required to combine with the iodine of the iodoform.

The work with less than an initial effective 0.045 *N* silver nitrate solution showed that accurate quantitative results cannot be obtained in a reasonable length of time. (Compare Experiments 11–13, Table 1, inclusive, with Experiments 9–12, inclusive, Table 2).

When an alcoholic solution of iodoform is left to stand a considerable length of time (overnight) before the silver nitrate is added, a low result is obtained, apparently due, in part at least, to the liberation of iodine (Experiments 14–17 inclusive, Table 1).

To summarize, the preliminary quantitative experiments (Table 1) show clearly that an increase in accuracy would be attained by using per 0.1313 gram of iodoform approximately 20 cc. of aqueous 0.1 *N* silver solution (twice the silver theoretically required), 20 cc. of alcohol as a solvent for the iodoform, and 5 cc. of nitric acid (68 per cent).

Table 2 gives results of experiments in which the only variable is the length of the reaction period. In each experiment the sample of iodoform (0.1313 gram), dissolved in 20 cc. of alcohol, was treated with 20 cc. of 0.1 *N* silver nitrate solution (aqueous) and 5 cc. of nitric acid (68 per cent). With the exception of the length of the reaction period, all the details of

TABLE 2.

*Determinations of iodoform using different reaction periods**

(Results obtained by Associate Referee.)

EXPERIMENT NO.	REACTION PERIOD	IODOFORM FOUND	EXPERIMENT NO.	REACTION PERIOD	IODOFORM FOUND
	hours	*% by weight*		*hours*	*% by weight*
1	1/4	98.7	18	3	99.9
2	1/2	98.0	19	4	100.1
3	1/2	97.4	20	5	99.6
4	1/2	98.6	21	5	100.1
5	1/2	95.7	22	17	99.1
6	1/2	94.2	23	17	99.8
7	1	98.6	24	17	99.4
8	1	99.6	25	2	99.7
9	2	99.9	26	2	99.8
10	2	99.7	27	2	99.9
11	2	99.7	28	3	99.8
12	2	100.0	29	3	99.8
13	3	100.0	30	17	99.2
14	3	100.1	31	17	99.2
15	3	99.7	32	18	99.1
16	3	100.0	33	18	99.4
17	3	99.7			

* For experiments 1–24, inclusive, volumetric method was used For experiments 25–33, inclusive, gravimetric method was used

the procedure used were those of the proposed method. Experiments 9–18 inclusive, and 25–29, inclusive, in which the reaction period was 2 or 3 hours, were made according to the method which subsequently was subjected to collaborative study. The results reported by the collaborators are given in Table 3.

DISCUSSION OF RESULTS (TABLE 2)

These experiments show clearly that two or three hours is a very satisfactory reaction period. Although about 95 per cent or more of the iodoform will react with silver nitrate in 15 minutes, uniformly satisfactory quantitative results were not obtained in less than 2 hours. The equivalence-point is not attained as readily as in the case of an alkali halide with silver nitrate. This would be expected because iodoform is not an electrolyte.

TABLE 3.

Results reported by collaborators.

COLLABORATOR	ALTERNATIVE METHOD USED			
	Volumetric*		Gravimetric	
	per cent		*per cent*	
Leslie Hart	100.28		99.40	
	100.28		99.67	
	100.28		99.73	(av. 99.6)
	100.04	(av. 100.2)		
Andrew G. Buell	—		99.67	
			99.41	(av. 99.5)
Llewelyn Jones	99.72		99.56	
	99.64	(av. 99.7)	99.68	
			99.72	(av. 99.6)
Thos. C. Dunn	99.95	(av. 100.0)	99.71	
			99.80	(av. 99.8)
Wm. C. Cavett	99.84		99.68	
	99.89	(av. 99.9)	99.57	(av. 99.6)
Irwin S. Shupe	99.94		—	
	99.86	(av. 99.9)		
Robert D. Stanley†	99.98		99.84	
	99.67		99.47	
	99.53	(av. 99.7)	99.51	(av. 99.6)
N. E. Freeman‡	100.0		—	
	99.6			
	99.6			
	99.6	(av. 99.7)		
C. K. Glycart	—		99.86	
			99.86	(av. 99.8)
Frank C. Sinton	99.58		—	
	99.47	(av. 99.5)		
Joseph A. Batscha	99.76		—	
	99.59			
	99.85			
	99.65			
	99.61	(av. 99.7)		
L. E. Warren	—		99.09	
			99.04	
			99.21	
			99.10	
			99.58	(av. 99.2)

* 0.05 *N* ammonium thiocyanate and non-alcoholic factor was used except where noted.
† Standardised 0.05 *N* ammonium thiocyanate in initial 50 per cent alcohol.
‡ 0.1 *N* ammonium thiocyanate used.

The low results in Experiments 30–33, inclusive, no doubt are due to peptization or the changing back again of a very small quantity of the flocculated silver iodide into colloidal solution.

GENERAL REMARKS

Although the proposed method is simple, to obtain accurate results the details must be followed reasonably close. The chemist cannot take the same liberties regarding acidity, quantity of excess of silver nitrate, and length of the reaction period that he can in the ordinary determination of a halogen with silver.

It is interesting to note that iodoform is less readily saponified by alcoholic potassium hydroxide than is chloroform or carbon tetrachloride. On the other hand, iodoform in alcoholic solution will readily react quantitatively with aqueous 0.1 *N* silver nitrate in the presence of nitric acid, while chloroform will not react under similar conditions, or even when 0.5 *N* silver nitrate solution is used and left to stand for an extended time.

As compared with a 0.1 *N* ammonium thiocyanate solution, a 0.05 *N* solution is specified in the proposed method so as to increase the accuracy of the titration. One drop (0.05 cc.) of a 0.1 *N* solution = 0.00065 gram of iodoform, or 0.26 per cent on the basis of a 0.2500 gram sample of iodoform.

When standardizing the 0.05 *N* ammonium thiocyanate solution against the 0.1 *N* silver nitrate solution and using a volume of alcohol equal to the 0.1 *N* silver nitrate solution (simulating the conditions of the proposed method) the factor is approximately 0.003 higher than when standardized in non-alcoholic solution. This means that when the excess of 0.1 *N* silver nitrate solution is equal to the 0.1 *N* silver nitrate solution consumed (as specified in the method) and the non-alcoholic factor for 0.05 *N* ammonium thiocyanate solution is used, the error is plus 0.3 per cent.

PROPOSED METHOD FOR THE DETERMINATION OF IODOFORM SUBMITTED FOR COLLABORATIVE STUDY

REAGENTS

(a) *Silver nitrate solution.*—0.1 *N*.

(b) *Ammonium thiocyanate solution.*—0.05 *N*. Standardize against 0.1 *N* silver nitrate solution, using an equal volume of alcohol and 3 cc. of ferric ammonium sulfate as indicator.

(c) *Nitric acid.*—68 per cent.

(d) *Ferric ammonium sulfate indicator.*—Dissolve 8 grams of ferric ammonium sulfate in sufficient water to make 100 cc.

(e) *Alcohol.*—(95 per cent by volume).

DETERMINATION

Weigh accurately about 0.25 gram of iodoform and transfer quantitatively to a 200 cc. Erlenmeyer flask. Add 40 cc. of alcohol, swirl gently until iodoform is dissolved, filter, if necessary, and immediately add 40 cc. of 0.1 *N* silver nitrate and 10 cc. of concentrated nitric acid.

Swirl gently for about 5 minutes and allow to stand at room temperature for 2–3 hours. Swirl occasionally as an aid in flocculating the silver iodide.

Titrate the excess 0.1 *N* silver nitrate with 0.05 *N* ammonium thiocyanate, using 3 cc. of ferric ammonium sulfate as indicator. 1 cc. of 0.1 *N* silver nitrate = 0.01313 gram of iodoform. Or, filter, collecting the silver iodide on a dried and accurately weighed Gooch crucible, wash with water and finally with alcohol, and dry to constant weight at about 125°C. 1 gram of silver iodide = 0.5590 gram of iodoform.

In the method actually submitted to the collaborators, the use of alcohol in the standardization of the 0.05 *N* ammonium thiocyanate solution was not specified. The alcohol has been included in the proposed method because of the experience of several collaborators and the associate referee. Furthermore, the conditions more nearly simulate those of a determination of iodoform made by the proposed method.

One of the collaborators suggested that the words "a mixture consisting of" be inserted after the word "add" in the fourth line of the text of the method. After adding a mixture consisting of the 0.1 *N* silver nitrate solution and the nitric acid in conforming to this suggestion, it would be necessary to wash the container in case the alternative volumetric procedure were used. Such washing would dilute the reaction mixture, which is not desirable, and as shown experimentally such dilution might lead to inaccurate results. This suggestion was not incorporated in the proposed method because very satisfactory results were obtained by the collaborators and associate referee when the method as given was followed.

COMMENTS BY COLLABORATORS

Leslie Hart.—The titration is quicker and for that reason is more desirable.

Andrew G. Buell.—A very practical method.

Llewelyn Jones.—The method produces very satisfactory results.

Thos. C. Dunn.—Appears to be a very good method. I believe the ammonium thiocyanate solution should be standardized in a 50 per cent alcohol solution as the end point is slightly different than when standardized in water.

Wm. C. Cavett.—The ammonium thiocyanate solution was standardized in aqueous solution, which may account for slightly higher results than those obtained by the gravimetric method.

N. E. Freeman.—I have no adverse comments to make. The end point is sharp and characteristic.

Frank C. Sinton.—The method is rapid and seems satisfactory.

C. K. Glycart.—The method is direct and rapid.

Joseph A. Batscha.—The method is convenient and quite satisfactory.

L. E. Warren.—I would suggest that in the fourth line of the text of the method, after the word "add," the words, "a mixture consisting of" be inserted.

SUMMARY OF RESULTS OBTAINED BY COLLABORATORS AND ASSOCIATE REFEREE

COLLABORATORS	VOLUMETRIC	GRAVIMETRIC
Number reported:	9	8
Determinations reported:	25	22
Range:	99.53–100.28%	99.04–99.84%
Average:	99.8%	99.5%
ASSOCIATE REFEREE	**VOLUMETRIC**	**GRAVIMETRIC**
Determinations made:	10	5
Range:	99.7–100.1%	99.7–99.9%
Average:	99.9%	99.8%

CONCLUSION

A new method, simple, rapid and accurate for the quantitative determination of iodoform, has been developed.

The procedure depends upon the reaction of iodoform in an alcoholic solution with silver nitrate (an excess of 0.1 *N* aqueous solution) in the presence of nitric acid *without the use of applied heat.* So far as could be learned this procedure has not previously been reported.

For very accurate work the standardization of the 0.05 *N* ammonium thiocyanate solution should be carried out under conditions simulating, as regards alcohol content, an actual determination of iodoform. (When the non-alcoholic factor is used the error is about plus 0.3 per cent.)

RECOMMENDATIONS[1]

It is recommended—

(1) That the method developed by the associate referee for the determination of iodoform be adopted as a tentative method with the view to adoption as official.

(2) That work on the quantitative determination of iodoform in mixtures be undertaken.

[1] For report of Subcommittee B and action of the association, see *This Journal*, 14, 54 (1931)

CONTRIBUTED PAPERS

THE ASSAY OF TABLETS OF RESIN OF PODOPHYLLUM*

By L. E. WARREN (U. S. Food and Drug Administration, Washington, D. C.).

Resin of podophyllum has been used in medicine for nearly a century.[1] As was noted in a previous paper,[2] the name "resin of podophyllum" is not correct. The preparation is not a true resin, but a mixture of substances, among which are podophyllotoxin, podophyllo-resin and quercetin. Resin of podophyllum is conveniently administered in pill or tablet form, and it is frequently mixed with other laxatives such as aloe, extract of colocynth, extract of cascara, extract of leptandra, resin of jalap, resin of ipomea, etc. This study considers tablets of resin of podophyllum without admixture with other laxatives.

In 1927[3] the writer compared several processes for determining resin in podophyllum and came to the conclusion that the Jenkins process, slightly modified,[4] was the most satisfactory of those tried. Briefly, this process consists of shaking a solution of the resin in alcohol and chloroform with 0.6 per cent hydrochloric acid. The chloroform-soluble part is again washed with 0.6 per cent hydrochloric acid, and after suitable treatment it is weighed. The U.S.P.X method gave results that were much too high, and it was shown to be unreliable in other ways. Last year, after trying several methods for the assay of resin of podophyllum, the writer favored[2] an adaptation of the Jenkins process. In that study ten specimens of the resin were assayed by this method. Other assay processes were considered, but they were discarded in favor of the Jenkins method. Moisture, ash, and the ether-soluble, chloroform-soluble, and alcohol-soluble portions were also determined. The results of that examination showed that the U.S.P.X methods for determining the ether-soluble and chloroform-soluble fractions of resin of podophyllum are unsatisfactory. Some inequalities in the composition of the resin of podophyllum supplied were also noted.

Since resin of podophyllum is administered chiefly as tablets and no method of assay appeared to be available for this form, the desirability of having such a process became apparent. Tablets and pills of resin of podophyllum usually contain starch, starch paste, talc, acacia, and liquid petrolatum as binders, fillers, excipients, etc. Therefore, in considering methods for assaying these tablets the first problem is that of extraction

* Read at the 81st Meeting of the American Chemical Society, Indianapolis, Indiana, March 31, 1931 and published here through the courtesy of Industrial and Engineering Chemistry.

[1] King, *N. Y. Philosoph. Med. J.*, 1, 157 (1844).
[2] *This Journal*, 13, 117 (1930).
[3] *Ibid.*, 10, 272 (1927).
[4] *J. Ind. Eng. Chem.*, 6, 671 (1914).

of the resin from the inert materials. Accordingly, several brands of tablets (and granules) of resin of podophyllum were obtained from the respective manufacturers, and a study of analytical methods was made with the material supplied.

The subject was considered under two phases: (a) extraction of the resin from the tablet material; and (b) determination of the resin in the extract.

CHOICE OF SOLVENTS

It is known that alcohol and acetone are good solvents for resin of podophyllum, and that chloroform, ether, benzene, and petroleum benzin are poor solvents. For these studies alcohol was selected as the solvent.

A quantity of the powdered tablet material was treated with alcohol in various ways, such as maceration, percolation, automatic extraction, etc. As a result of preliminary trials two extraction procedures were devised. These were submitted to several analysts for trial and opinion, and were also applied to some of the material at hand. These procedures are described as follows:

Extraction Procedure I

Weigh a sufficient quantity of the powdered tablet material to represent approximately 1 gram of resin of podophyllum. Place the mixture in a small beaker and macerate with 50 cc. of alcohol for 20 minutes with occasional agitation. Decant the supernatant liquid through a Gooch crucible or other suitable filter. Wash the insoluble matter into the crucible (or filter) with small quantities of alcohol and wash the residue with alcohol until the washings are colorless and no longer precipitate when mixed with several volumes of water. Transfer the alcoholic solution to a 100 cc. graduated flask, make up to the mark with alcohol, and mix well.

Extraction Procedure II

Weigh a sufficient quantity of the powdered tablet material to represent at least 1 gram of resin of podophyllum, place it in a fat-free thimble, and extract with alcohol in a Bailey, Havenhill, or Soxhlet extractor until completely exhausted of resin. Transfer the alcoholic solution to a 100 cc. graduated flask, cool it to room temperature, make up the volume to the mark with alcohol, and mix well.

Procedure I was discarded because with some brands of tablets filtration was too slow, and with one brand (Specimen D) the extraction was shown to be incomplete.

In working with Procedure II it was observed that some specimens of the powdered tablet (or granule) material had a tendency to mass together in the thimble, thus rendering extraction slow. It was found that this could be remedied by mixing the powdered material with twice its weight of fine, washed sand. In one test powdered potassium sulfate was tried as a diluent, but the extraction of the resultant mixture was not so satisfactory as that in the experiments in which sand had been used. Four samples of tablets of resin of podophyllum were sent to each of four collaborators with the request that they be extracted by each of the two pro-

cedures and that the alcoholic extract be assayed by the modified Jenkins process.[1]

As a result of experimental trials and in consideration of the results and comments obtained from the collaborating analysts, the second method of extraction was modified as given below and most of the analyses reported in Table 2 were made by this procedure. The process is as follows:

Extraction Procedure III

Weigh into a beaker a sufficient quantity of the powdered tablet material to represent approximately 0.75 gram of resin of podophyllum; add 10 grams of fine, washed sand, mix well, and transfer the mixture to a 30 cc. Gooch crucible. Pour 25 cc. of alcohol in small portions through the crucible, collecting the washings in a 100 cc. graduated flask. Allow the crucible to drain. Place it in a Bailey or a Soxhlet extractor and extract with 60 cc. of alcohol until the residue is completely exhausted of resin. Transfer the alcoholic solution to the graduated flask, using alcohol in small portions for washing, cool the contents of the flask to room temperature, and make up to the mark with alcohol.

The Jenkins process, slightly modified for the determination of resin of podophyllum was then applied to the alcoholic extracts of the tablets. The method used is as follows:

Assay

Measure 10 cc. of the tincture, prepared by the procedure described above, into a separator; add 10 cc. of chloroform and 10 cc. of 0.6 per cent hydrochloric acid (2 cc. of hydrochloric acid in 100 cc. of water). Shake the mixture thoroughly and allow it to separate. Draw off the lower layer into another separator, and repeat the extraction of the liquid in the first separator three times, using 15 cc. of a mixture of one volume of alcohol and two volumes of chloroform each time, and adding these extractions to the extraction in the second separator. Shake the combined extractions with 10 cc. of 0.6 per cent hydrochloric acid and allow the mixture to separate. Draw off the lower layer into a weighed beaker or Erlenmeyer flask and repeat the extraction of the acid liquid three times, using 15 cc. of fresh alcohol-chloroform mixture each time. Evaporate the combined chloroform extractions, taking care to rotate the container in an inclined position as the last portions of the solvent are dissipated, and dry the residue to constant weight at 80°C.

A mixture simulating ground tablet material was prepared from 2.5 grams of commercial resin of podophyllum (the anhydrous resin content of which was known), 6.0 grams of washed starch, 1.0 gram of lactose, and 0.5 gram of talc. To insure thorough mixing, the powder was passed through a sieve three times. The sample of resin of podophyllum contained 3.15 per cent of moisture, 0.25 per cent of ash, and 0.5 per cent of alcohol-insoluble material and assayed 95.1 per cent of anhydrous resin by the Jenkins method. The proportions soluble in ether and in chloroform were not determined, as these factors have been shown[2] to be of little consequence in evaluating resin of podophyllum. Calculation indicated that the synthetic mixture should contain 23.77 per cent of anhydrous resin. This mixture was extracted by Procedure III, and the solution was assayed for

[1] *J. Ind. Eng. Chem.*, 6, 671 (1914).
[2] *This Journal*, 13, 117 (1930).

resin by the modified Jenkins method previously described. The results were 23.53, 23.96, 23.53 and 23.53 per cent of resin. They are equivalent, respectively, to 99.0, 100.8, 99.0, and 99.0 per cent of theory for anhydrous resin.

The results obtained by two collaborators from the known mixture and from the tablet specimen that had been especially prepared are given in Table 1.

TABLE 1.

Results of analysis of preparations containing resin of podophyllum.

COLLABORATOR		KNOWN MIXTURE		SPECIAL TABLET	
	Resin Content (anhydrous)	*per cent* 23 775		*per cent* 22 835 (calculated)	
A	Resin found (anhydrous)	23.53	23.53	24.05	22.87
		23.96	23.53	22.47	24.47
				23 08	
	Recovery (per cent of theory)	99.00	99.00	105.32	100.15
		100.81	99.00	98 40	100.11
				101.07	
B	Resin Content (anhydrous)	23.775		22.835	
	Resin Found (anhydrous)	22.53		23.44	
		22.83		23 49	
	Recovery (per cent of theory)	94.78		102.65	
		96.03		102.86	
		94.21			

Examination of Table 1 shows that the assay method used gives results in reasonably close agreement with the quantities of resin in the known mixture and also that the findings upon the tablet that had been especially made agree fairly well with the calculated composition.

During these studies residues that could be brought to constant weight only after long continued heating were encountered occasionally, and it was suspected that they occluded moisture or alcohol. It was found that these residues could be brought to constant weight by adding about 1 cc. of dehydrated alcohol and repeating the evaporating and heating. Accordingly the procedure was adopted as a routine by adding 1 cc. of dehydrated alcohol to the residue as soon as all the original solvent appeared to be evaporated. The added solvent was then evaporated and the residue dried in the usual way.

Eight specimens of tablets of resin of podophyllum and one specimen of granules of the resin were obtained from the manufacturers. One of these specimens had been prepared especially for this investigation from a good commercial grade of resin of podophyllum. A specimen of the resin from which this brand of tablets had been prepared was also furnished by the manufacturer. This specimen contained 2.5 per cent of moisture, 0.22 per cent of ash, 0.37 per cent of alcohol-insoluble matter, and assayed 92.1 per cent of resin by the modified Jenkins process. The proportions soluble in ether and in chloroform were not determined. Calculation indicated that this specially prepared tablet should contain about 22.84 per cent of anhydrous resin.

Of the nine specimens of tablets and granules received, two were from one manufacturer but were from different lots. The declared content of these preparations varied from $\frac{1}{8}$ to $\frac{1}{2}$ grain. All the specimens were subjected to the assay processes described above, that is, extraction by Procedure III and assay for resin by the modified Jenkins process. One of the specimens (B) gave results that were much higher than expected. From the appearance of the weighed residue it was suspected that liquid petrolatum had been used as a lubricant on the tablet machine and that this substance had been carried through the assay process and weighed with the resin. This suspicion was confirmed by washing the weighed residue with petroleum benzine and decanting the solution through a filter into a beaker. Evaporation of the solvent left a residue that had the appearance of liquid petrolatum. The washed residues were then dried and weighed. For specimen B the weights of the washed residues were considered as the correct values in the assay. The presence of liquid petrolatum in this tablet was further confirmed by correspondence with the manufacturer. The values corrected for liquid petrolatum are also recorded in Table 1, and the findings for the several brands of tablets are given in Table 2.

SUMMARY

A method is described (Procedure 3) for extracting resin of podophyllum from tablet material and for determining resin in the extract. The recommended method is simple and is believed to evaluate the preparation sufficiently well for control work and for purposes of comparison. Nine brands of tablets of resin of podophyllum were examined. These tablets ranged in resin content (anhydrous) from 83 per cent to 124 per cent of the amounts of resin claimed. Seven of the brands ranged from 90 per cent to 112 per cent of the amounts claimed to be present.

ACKNOWLEDGMENTS

Thanks are due to a number of pharmaceutical manufacturers for contributing material for this study. The writer is particularly indebted to

TABLE 2.
Analyses of tablets of resin of podophyllum.

	SAMPLE	A	B	C	D	E	F*	G*	H	ESPECIALLY MADE
	Claim (gram per tablet)	¼ grain (0.0162)	¼ grain (0.0162)	⅕ grain (0.0108)	¼ grain (0.0162)	⅕ grain (0.0181)	½ grain (0.0324)	¼ grain (0.0162)	¼ grain (0.0162)	¼ grain (0.0162)
Analyst A	Anhydrous resin (per cent)	12.00 11.36 11.32	13.09 13.25	45.81 43 08 45.29	20.23 20.23	10.12 10.13	28.59 28.59	13.50 13.43	19.12 19.35	24.05 24.86 24.87 24.47
	Anhydrous resin (gram per tablet)	0.01712 0.01620 0.01615	0:01530 0.01547	0.01035 0.00973 0.01023	0.01572 0.01572	0.01130 0.01131	0.0269 0.0269	0.01819 0.01810	0.01407 0.01423	0.01577 0.01630
	Percentages of claim	105.4 100.0 99.7	94.34 95.57	95.8 90.1 94.7	97.07 97.07	124.2 124.1	83.03 83.03	112.3 111.7	86.85 87.87	97.33 100.62 101.67 99.80
Analyst B	Anhydrous resin (per cent)	11.80 11.76 11.32		43.81 43.81	21.03 20.93	10.49 10.44			18.90 19.08	23.44 23.49
	Anhydrous resin (gram per tablet)	0.01682 0.01680 0.01615		0.0099 0.0099	0.01640 0.01632	0.01039 0.01037	0.0272 0.0270	0.01188 0.01198	0.0139 0.0140	0.01537 0.01540
	Percentage of claim	103.8 103.5	99.7	91.7 91.7	101.2 100.8	128.25 128.00	83.8 83.3	109.7 110.9	85.83 86.64	94.86 95.06
	Manufacturer's findings (percentage of claim)				98.96† 97.90‡	115.4 116.2	61.27 61.57	108.6 109.8		91.5

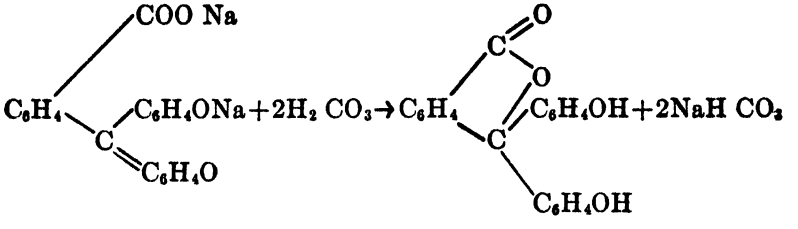
* Samples F & G were from the same manufacturer.
† Average of 8 analyses.
‡ Average of 4 analyses.

the Zemmer Company for preparing a special batch of tablets. Thanks are also due to the School of Pharmacy of Purdue University for aid in the collaborative assays and to William R. Carter for carrying out a number of the routine determinations.

A COLORIMETRIC METHOD FOR THE DETERMINATION OF CARBON DIOXIDE*

By E. M. EMMERT (University of Kentucky, Lexington, Ky.)

When carbon dioxide is shaken with a solution of the sodium salt of phenolphthalein the red color diminishes in proportion to the amount of CO_2 present. No doubt the change in color is due to the formation of sodium bicarbonate, which removes the sodium from the colored salt and leaves the phenolphthalein colorless. This reaction is shown in the following equation:



The measurement of this diminution in color may be used to indicate the amount of CO_2 introduced. The only precaution necessary is to keep other acidic or basic substances from entering with the CO_2. These substances may be eliminated by bubbling the gas through sulfuric acid solution (1 per cent). The following method was found to be rapid and accurate:

REAGENTS AND PREPARATION OF SOLUTIONS

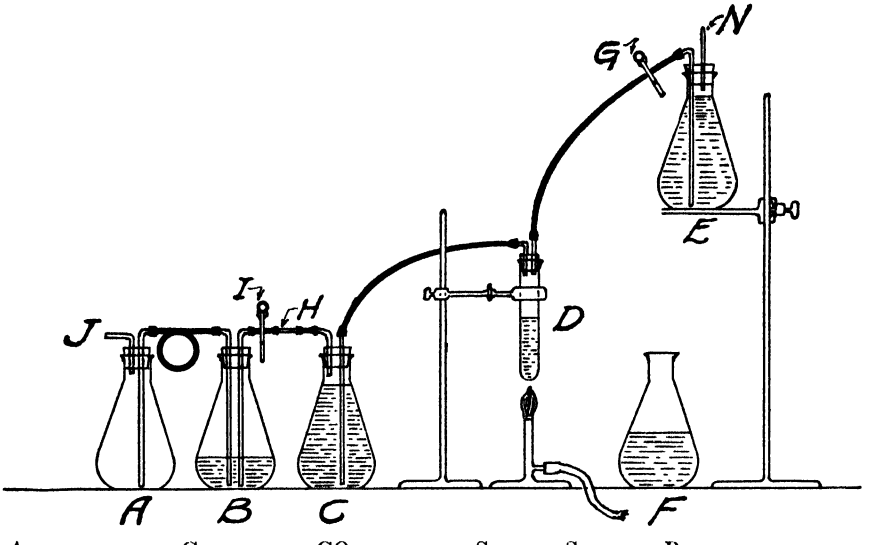
(a) *Alcohol neutral to phenolphthalein.*—To 95 per cent alcohol add a few drops of 0.5 per cent phenolphthalein solution and then normal sodium hydroxide, dropwise, until a faint pink color persists.

(b) *Solutions of the colored salt of phenolphthalein.*—Prepare different strengths of solution for different amounts of CO_2 as follows:

FOR THE ESTIMATION OF THE FOLLOWING QUANTITIES OF CO_2	USE 100 CC. OF THE FOLLOWING STRENGTHS OF THE SODIUM SALT OF PHENOLPHTHALEIN SOLUTION DESCRIBED BELOW
mg	*N*
2– 9	0.0025
9–18	0.0050
18–35	0.0100
35–50	0.0150
50–70	0.0200
70–90	0.0250
90–120	0.0300

* The investigation reported in this paper is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

Dissolve slightly more than the required amount of phenolphthalein to make 2 liters in 95 per cent alcohol made neutral to phenolphthalein and make up to 1 liter with neutral alcohol. Dissolve the amount of sodium hydroxide required in distilled water and make to 1 liter. Pour the two solutions together. For accurate work the sodium salt of the phenolphthalein solutions should be standardized. This may be accomplished by titrating against standard acid or by using the known carbonate solution [Reagent (c)] and proceeding as directed in the procedure. Keep the solutions from the air.



APPARATUS FOR COLLECTING CO_2 OVER THE SODIUM SALT OF PHENOLPHTHALEIN

(c) *Known carbonate solution.*— Dissolve 12.05 grams of pure anhydrous sodium carbonate in 200 cc. of distilled water and make to 500 cc. One cc of this solution contains 10 mg. of CO_2.

(d) *Sulfuric acid solution.*—1 per cent. Dilute 10 cc. of concentrated sulfuric acid to 1000 cc.

(e) *Sulfuric acid solution.*— 50 per cent. Add 500 cc. of concentrated sulfuric acid to 500 cc. of water.

PROCEDURE

Put the unknown (liquid or solid) containing 5–120 mg. of carbon dioxide into test tube *D* (see figure), which should be of large size for large samples. Fill *D* to within 10–15 cc. of the top with water. Put 100 cc. of the appropriate concentration of Reagent (b) into flask *B*. *C* should be filled to within 10–20 cc. of the top with 1 per cent sulfuric acid. *F* contains Reagent (e) Stopcock *I* should be open. Make certain all connections are air-tight. From *F* blow in 10–15 cc. of Reagent (e) by placing the stopper and tubes from *E* in *F* and blowing at *N*, at the same time opening *G*. Close *G* and replace stopper and tubes in *E* without destroying the siphon started. If much CO_2 is present, the acid should be introduced slowly.

Heat *D* until the liquid boils several seconds, taking care that the solution does not boil over into *C* to any extent. Withdraw the flame, immediately open *G*, and allow the gases to be forced from *D* and *C* by Reagent (d) from *E* until the liquid reaches the glass tube *H* (4–5 inches long). As soon as the liquid appears in *H*, close

I tightly. Be careful not to allow any acid solution to get into *B*. The connection at *H* and the other tubes leading to *B* should be washed after each determination as a trace of acid might affect the results. Disconnect *B* from *C* at *H*. Lower *B* below *A* and shake *B* vigorously at intervals until the solution in *B* stops changing color. If the solution in *B* gets colorless or nearly so and considerable colored solution has

Results of determinations of carbon dioxide in known amounts of sodium carbonate.

CO_2 PRESENT *mg.*	CO_2 FOUND *mg.*	ERROR *per cent*
2	2.20	10.0
3	2.98	−0.7
4	4.27	6.7
5	5.30	6.0
5	4.98	−0.4
5	5.00	0.0
7	6.72	−4.0
7.4	7.88	6.5
10.0	9.90	−1.0
10.0	10.00	0.0
11.1	11.10	0.0
14.8	15.72	6.2
15.0	15.00	0.0
18.5	18.65	0.8
18.5	18.50	0.0
18.5	18.50	0.0
18.5	17.98	−2.8
18.5	17.99	−2.8
20.0	20.00	0.0
22.2	21.72	−2.2
22.2	21.65	−2.5
40.0	50.00	0.0
50.0	50.00	0.0
60.0	61.40	2.3
70.0	69.40	−0.9
80.0	79.40	−0.7
90.0	90.00	0.0
100.0	100.00	0.0
100.0	112.70	2.4
120.0	118.70	−1.1

been forced into *A*, draw most of the colorless solution from *B* into *A* by applying suction at *J*. When most of the solution in *B* is drawn out, release the suction and allow the vacuum created in *B* to draw back as much of the solution as it will. If the quantity of solution in *B* is small, it will likely be decolorized several times, making it necessary to repeat the mixing of the solution by suction. If the solutions in both *A* and *B* get colorless or nearly so, either there was too much CO_2 in the sample or some acid was allowed to get into *B*.

After the color has stopped changing, remove the tubes and pour the solutions in *A* and *B* together and mix. Compare the color of the mixed solutions with the original color of the particular strength of reagent used.

The quantity (mg.) of carbon dioxide in the unknown is found by solving the following equation for *X*:

$$X = Y - \frac{YR}{U},$$

Where Y = the theoretical or found mg. of CO_2 equivalent to 100 cc. of the concentration of sodium salt of phenolphthalein solution used.

R = the original colorimetric reading of the sodium salt of phenolphthalein solution.

U = the colorimetric reading of the sodium salt of phenolphthalein solution after it has been shaken with the CO_2.

If the theoretical value for Y is not considered accurate, the actual value may be found by titrating against standard acid or by using a known volume of Reagent (c) in the place of the unknown in the above procedure. X then becomes known and equals the mg. of CO_2 introduced by Reagent (c). In this case the equation is solved for Y.

The CO_2 in a gas may be determined by introducing a known volume of the gas, measured at a known temperature and pressure, over the sodium salt of phenolphthalein solution by displacement. Proceed as in the foregoing instructions. If the amount of CO_2 in a gas is small, it should first be caught in concentrated sodium hydroxide in the usual ways.

RESULTS

Results of determinations of known amounts of CO_2 are given in the table. An error of 0.1–0.2 mg. of CO_2 may be expected from the CO_2 in the air enclosed in the shaking flasks. It is not significant in large amounts of CO_2, but is in amounts of 2–5 mg. This likely accounts for the larger percentage of error on small amounts of CO_2 in the table.

BOOK REVIEWS

Food Analysis. By A. G. WOODMAN. Third edition. 557 pages, 110 figures. McGraw-Hill Book Company, Inc., New York, 1931. Price $3.50.

The plan of the book is essentially the same as that of the second edition. Some methods have been revised to conform with those adopted by the Association of Official Agricultural Chemists. The book was developed from courses given in food analysis. The author states: "Because the primary intention has been to write a book of the character outlined no effort has been made to include a great variety of food materials, nor necessarily those of greatest economic importance or which are most widely used. Certain typical foods have been selected to illustrate important methods of attack or characteristic methods of food analysis. In a word, the book has been written and the material selected primarily for the undergraduate student of analytical chemistry rather than for the practicing chemist." The author has accomplished his object remarkably well.

There are eleven chapters devoted to the following topics: General Methods, The Microscopical Examination of Foods, Food Colors and Preservatives, Milk and Cream, Edible Fats and Oils, Carbohydrate Foods, Cocoa and Chocolate, Spices, Cider Vinegar, Flavoring Extracts, and Alcoholic Foods. The chapters on Fats and Oils, Carbohydrate Foods and Alcoholic Foods are treated most extensively. The detection and identification of artificial colors is treated extensively because "this part of the work frequently causes the student some difficulty and adequate discussions of it are hard to find" and because ". . . it affords excellent training in the detection of minute quantities of material through a systematic procedure." The sections dealing with alcoholic beverages purposely were not revised.—H. R. KRAYBILL

An Introduction to Biochemistry. By ROGER J. WILLIAMS. 501 pages. D. Van Nostrand Company, Inc. New York, 1931.

In this book the author has "attempted to present an outline embodying the more salient features of the subject of biochemistry as it appears in its present state of development. That the treatment is only an outline has beem emphasized in the various discussions and by reference to numerous additional topics at the ends of the chapters." It is primarily prepared for the class room as evidenced by the lack of specific references to the literature. However, this does not particularly detract from the value of the book as the material is presented in a clear and interesting style. The author has endeavored to attract students "to the possibilities of research in the numerous fields in and related to biochemistry."

The material presented is briefly outlined by the author to include: (1) Composition of the organisms, (2) Foods material required by the organisms, (3) Transformation of these food materials into materials composing the organisms. In discussing the chemical transformation occurring within the organisms the author states that, "The single celled organisms are chosen because of their simplicity, the green plant is chosen because of its tremendous importance to man and all life," and, "Mammals represent the most highly developed form of life and the discussions of their metabolism is, for obvious reasons, the most extended." The scope of the book is indicated by the following section headings: I, Composition of Organisms; II, Nutritional Requirements of Organisms; III, Mechanism Used by Organisms in General for Promoting and Regulating Chemical Change; IV, Metabolism of a Single Cell; V, Metabolism in Green Seed Plants; and VI, Metabolism in Mammals. Each section is subdivided into chapters of which there are twenty-seven. Appended is a section of "Suggested Laboratory Experiments," which number fifty-seven. Concerning

these the author states: "The material in this section is obviously not a comprehensive collection of experiments, and the instructor may wish to choose some material from other sources."

It is apparent that the author has endeavored to meet the need of a broad text on biochemistry suitable for students in medicine, biology, home economics, and agriculture, as well as for those students primarily interested in the field of chemistry. As a text, it appears to be admirably suited for undergraduate courses in general biochemistry.—SIGFRED M. HAUGE



ROBERT SILVER HILTNER, 1873-1931

ROBERT SILVER HILTNER

In the death of Robert Silver Hiltner, which occurred on July 5, 1931, at his home in Berkeley, California, scientific agriculture lost an earnest, able worker. As technologist for the California Dried Fruit Association his work brought him in close contact with the many phases of the dried fruit industry, and his advice and assistance were sought in many quarters. For many years he had been vitally interested in the various problems presented in the processes of the drying of fruits, and he had made important contributions to the knowledge and literature on the subject. The great adventure came to him at the peak of his activity and achievement, and in his death the entire fruit industry of California will experience a distinct loss.

Born in Lincoln, Nebraska, June 12, 1873, Mr. Hiltner received his early education in the public schools of his home town. In 1890 he entered the University of Nebraska, where the degrees of B.S. and M.A. were conferred upon him. After his graduation in 1894 he continued in the University as instructor in general and technical chemistry until 1903. For three years during that period he served also as assistant chemist in the Nebraska Agricultural Experiment Station.

For several years after leaving the university changes in fields of activity came rather rapidly to Mr. Hiltner. He was employed as chemist for the American Beet Sugar Company in California, chemist in the office of the Supervising Architect of the Treasury Department in Washington, and chemist and drug examiner in the U. S. Customs Service in Chicago. In 1907 he became a member of the Bureau of Chemistry of the U. S. Department of Agriculture and was assigned to duty in the Chicago laboratory as assistant to Dr. A. L. Winton, who was in charge of the work conducted there in the enforcement of the food and drugs act. After serving a part of the year in Chicago, he was transferred, early in 1908, to the Denver laboratory, then under the direct supervision of Mr. A. E. Leach. On the death of Mr. Leach in 1911, Mr. Hiltner was chosen as his successor as Chief of the Denver laboratory, and he retained this position until 1920.

In 1920 the National Canners Association offered Mr. Hiltner the position of director of its cannery inspection service for the State of Colorado. Mr. Hiltner accepted this position and filled it with credit for four years. In 1924 he went to California to assume the duties in connection with the Dried Fruit Association, in which he was engaged at the time of his death.

During the period of his service in the Government, Mr. Hiltner cooperated actively with the Association of Official Agricultural Chemists. He served as Referee on Flavoring Extracts and devised a method for the determination of citral in lemon oil and flavors which bears his name and is the basis of the official method of the Association of Official Agricultural Chemists. He devoted considerable time to research work on the zinc content of oysters and other shell fish, and obtained valuable data which are incorporated in various publications.

Of the many problems to which Mr. Hiltner gave his earnest and conscientious endeavor, perhaps on none did he expend so much enthusiasm and hard work as on his investigations during the World War

and the trying period of adjustment which followed on the conservation of vast quantities of fruits and vegetables by the application of new and improved methods of dehydration, and probably none was of more economic importance. By reason of the results obtained in his experiments he was able to give valuable assistance and advice to firms installing dehydrating plants on a commercial scale. These important lines of research proved a valuable foundation for his later studies for the Dried Fruit Association on the various problems connected with the commercial drying of fruit.

In addition to membership in Sigma Xi, which he won because of his scientific attainments at the University of Nebraska, Mr. Hiltner held membership in the American Chemical Society and the American Society for Testing Materials.

In 1903 Mr. Hiltner was married to Miss May Crabtree of Lincoln, Nebraska. Miss Crabtree was a student in the University of Nebraska at the time Mr. Hiltner taught chemistry there. In fact she was a member of his chemistry class. In later years he was rather fond of declaring that his wife knew no chemistry and explained it by saying she must have had a remarkably poor teacher in that science. His widow and two children survive him.

There was nothing spectacular about Robert Hiltner. A man of quiet and unassuming manners, his rare qualities of heart and mind were known and appreciated only by those who were closely associated with him. When he took up his duties in the enforcement of the food and drugs act, his initiative, sound judgment and ready tact were made manifest in the early days of organization and adjustment when such qualities were essential to the success of the work. Unrelenting though he was, in his application of official policies, his discretion and quick humor saved many a situation that promised to be at least strained. Insistent on unqualified compliance with regulations, he, at the same time, recognized the other man's rights and respected his views. Mr. Hiltner demanded much of his official staff, but his quick recognition of a duty well done, and his uniform fairness and consideration gave zest to the work and created interest in its performance.

He had a great love for the out-of-doors, and in the mountains of Colorado he found ample opportunity to indulge his skill by a trout stream or at the wheel of his car.

Integrity that knew no compromise, steadfast devotion to truth as he saw it, fearlessness in the expression of his convictions, were traits characteristic of the man that commanded the respect of all who knew him. Added to these qualities were a gentle courtesy and a rare charm of manner that greatly endeared "Bob" Hiltner to all who were fortunate enough to be included in the circle of his friends.

LOUIS D. ELLIOTT

SECOND DAY

TUESDAY—MORNING SESSION

REPORT ON EGGS AND EGG PRODUCTS

By SAMUEL ALFEND (U. S. Food and Drug Administration, St. Louis, Mo.), *Referee*

Some work was done on all but two of the twelve methods recommended for study this year. A rather unusual situation exists this year in that *Methods of Analysis*, *A.O.A.C.* is to be issued shortly, and consequently it is desirable that methods which are likely to be dropped in the future be deleted now, and that the adoption of more promising methods be hastened. In correspondence with J. A. Le Clerc of the Revision Committee the referee has suggested various minor changes in several of the methods. These suggestions will be incorporated in this report.

The gathering of collaborative data on liquid eggs has always presented great difficulties owing to the precautions to be taken against spoilage and the necessity for immediate analysis. This year J. O. Clarke and L. C. Mitchell of the U. S. Food and Drug Administration conducted an intensive investigation into methods of egg analysis, including the collection of collaborative data, and they have very kindly made the results of their investigations available for the use of the referee and of Associate Referee Bornmann. The collaborative work was carried out under more carefully controlled conditions than usually obtain, and the results are considered reliable. The referees have drawn freely upon these data.

TOTAL SOLIDS

The tentative 98°C. vacuum-oven method, which was adopted as official (first action) last year, has proved to be accurate and reliable and to give concordant results in the same laboratory, but results from different laboratories on the same sample were lacking. These are now available on three samples. The first sample, a liquid whole egg, was sent out by Mitchell in a frozen condition. The results are considered to be remarkably good in view of the fact that when the samples arrived most of the bottles were found to be broken The total variation among five analysts (see Table 1) was 0.25 per cent, and the average deviation from the average value was 0.08 per cent. Mitchell's results on Sample 7573 indicate that freezing and thawing do not affect the moisture results.

A second sample of liquid whole egg (No. 7469) was prepared by Mitchell under aseptic conditions and sent out in sterilized containers without refrigeration. The variation in results on this sample was approximately the same as was found on No. 7573, the range being 0.30 per cent and the

average deviation 0.07 per cent. No. 7648, a sample of spray-dried egg yolk, was analyzed by seven collaborators. With the exception of results from one analyst the agreement is satisfactory, the total range being 0.23 per cent and the average deviation 0.06 per cent.

The vacuum-oven method is thus seen to be worthy of adoption as official (final action). There are several details which should be altered before the method is included in the new book. There is no record of comparative work with desiccants to justify the specification of calcium carbide or reignited quick lime. Calcium carbide is open to objection because of the liberation of acetylene. Reignited quick lime is not so efficient a desiccant as sulfuric acid. It is the belief of the referee that sulfuric acid is still the most widely used desiccant for most purposes. Dried egg solids are not extremely hygroscopic—not nearly so much so as flour, for instance—and the highest possible efficiency is not required of the desiccator in this case. With the many new desiccants on the market today, such as porous barium oxide, barium and magnesium perchlorates, etc., it should not be difficult for the analyst to prepare a satisfactory desiccator to hold the dried egg samples. It is therefore recommended that paragraph **(b)** under "Apparatus"[1] be changed to read: "**(b)** Air-tight desiccator.—Should contain a fresh, efficient desiccant."

The weight of moisture adsorbed on dishes of the size and material specified is no greater than the weighing error, and the elaborate directions for previous heating of the dishes are considered unnecessary It is therefore recommended that in line 2 of paragraph **(a)** under "Determination," the words "that previously has been dried at 98°–100°C., cooled in the desiccator, and weighed soon after attaining room temperature" be omitted. The same directions under paragraph **(b)**, Liquid or Frozen Eggs, should also be omitted.

ROUTINE AIR-OVEN METHOD

No work was done on this method. Previous collaborative work[2] has demonstrated that the results obtained by this method are not so satisfactory as those obtained by the vacuum-oven method. The air-oven procedure was introduced, presumably, to reduce the time and provide for those laboratories that do not have a vacuum oven. The method offers but little advantage over the vacuum oven method as regards time, as a reduction from 5 to 3 hours in drying time is of less consequence in an egg-drying plant or an official laboratory than in a flour mill. Adjusting the temperature of the oven often interferes with other work in the laboratory. As for the contention that many laboratories do not have a vacuum oven, this is becoming less and less true, and it is doubted if any official laboratory in the country now lacks a vacuum oven. Therefore, it is

[1] *This Journal*, **9**, 56 (1926).
[2] *Ibid*, **13**, 405 (1930).

recommended that the routine air-oven method[1] be dropped, and work on this method be discontinued.

SUGARS

The association recommended that methods for the determination of added sugars be studied. It was also recommended, under "Detection of Decomposition," that methods for determining reducing substances as dextrose be studied. It is highly desirable that the methods should be mutually adaptable.

Mitchell found that the method for reducing sugars used by Redfield and his co-workers[2] was not suitable for the determination of added sucrose because heating with acetic acid caused inversion of sucrose. He has proposed a method which produces concordant results (see Table 4) similar to those obtained by Redfield for reducing substances in whole eggs, and it seems to be adapted to the successful determination of added sugars. It is probable that the clarification of the solution, as described below, can be simplified with a little more study. The method follows:

DEXTROSE AND SUCROSE

REAGENTS

(a) *Sodium chloride solution.*—Dissolve 50 grams of sodium chloride in water and dilute to 1 liter.

(b) *Alcohol.*—95 per cent.

(c) *Phosphotungstic acid.*

(d) *Calcium carbonate.*

(e) *Sodium hydroxide solution.*—Dissolve 1 volume of 1+1 sodium hydroxide solution with 4 volumes of water.

(f) Powdered potassium chloride.

PREPARATION OF SOLUTION

Weigh accurately, by difference, approximately 25 grams of the well-mixed sample into a 250 cc. volumetric flask containing 50 cc. of the sodium chloride solution and 1 gram of calcium carbonate, add with continuous mixing 130 cc. of 95 per cent alcohol, let stand a few minutes for gas bubbles to rise to the surface, fill to mark with water, shake, and filter (18½ cm. folded filter). Transfer 150 cc. of the filtrate to a 250 or 400 cc. beaker, evaporate to 20–30 cc. to remove the alcohol, cool, wash with water into a 100 cc. volumetric flask, holding volume to 80–90 cc., add in small amounts dry phosphotungstic acid in slight excess to precipitate any proteins, mix vigorously, let stand a few minutes for gas bubbles to rise to the surface, fill to mark with water, shake, and filter. To the filtrate add sufficient dry powdered potassium chloride to precipitate the excess of phosphotungstic acid, filter, and test the filtrate for complete precipitation.

DETERMINATION

Reducing sugars direct.—Transfer 25 cc. of the prepared filtrate to a 400 cc. beaker, add 25 cc. of water, and proceed as directed on p. 190, **35**, *Methods of Analysis*, 1925. Calculate result as percentage of dextrose.

[1] *This Journal*, **9**, 57 (1926)

[2] U. S. Dept. Agr. Bur Chem Bull 846 (1920).

Reducing sugars invert.—Transfer 50 cc. of the prepared filtrate to a 100 cc. volumetric flask, add 5 cc. of strong hydrochloric acid, and allow the mixture to stand overnight. Exactly neutralize with the sodium hydroxide solution, cool to room temperature, and fill to mark with water. Transfer 50 cc. (or less) to a 400 cc. beaker, and proceed as directed on p. 190, 35, *Methods of Analysis*. Deduct the percentage of invert sugar obtained before inversion from that obtained after inversion and multiply the difference by 0.95 to obtain the percentage of sucrose.

It is recommended that Mitchell's method be studied next year with a view to its adoption as a tentative method for the determination of reducing sugars and of sucrose. This study should include the collaborative analysis of egg samples containing known amounts of added sugars.

UNSAPONIFIABLE MATTER

The association recommended that the tentative method for the determination of unsaponifiable matter be studied collaboratively with a view to its adoption as an official method. Owing to the rather wide discrepancies encountered in the work of 1928[1] and 1929[2] it was considered advisable to study the method thoroughly, so that the cause of these poor results might be ascertained. The referee has been able to obtain fairly concordant results in duplicate analyses when the lipoids checked, but he was unable to find the cause of the poor results obtained on collaborative analysis. The value of this method at the present time is not sufficient to justify more work on the present method. However, the method may turn out to be of value in the future in connection with incubated eggs, and it is therefore recommended that the studies on this method be continued. The lipoids obtained by the alcohol-chloroform extraction proposed by Mitchell offer a promising starting point for the saponification. Another possibility is suggested by the method Mancini[3] uses in extracting cholesterol from biliary calculi.

ASH

The preliminary work on which the present official method for ash[4] is based was performed on liquid and dried whole egg. The amount of magnesium acetate added was calculated to be just sufficient to combine with the acidic constituents of the yolk. The method specifies ashing at a low red heat. A check-up on the muffle used for this work makes it appear likely that the temperature used was 600°C. or slightly higher. Under these conditions, the difference between the amounts of magnesium oxide and magnesium carbonate in the blank and in the sample was considered negligible.

Clarke and Mitchell[5] conducted an extensive investigation on the ashing of yolks and whites separately and concluded that the magnesium

[1] *This Journal*, 12, 348 (1929).
[2] *Ibid.*, 13, 405 (1930).
[3] *Biochim. Terap. sper.*, 16, 9 (1929); *C. A.*, 23, 3726 (1929).
[4] *This Journal*, 12, 35 (1929).
[5] Unpublished

acetate fixative will not fix the chlorine, and that it is unsuited for egg white and probably unsuited for egg yolk. Although they did not use the exact procedure of the tentative method, their results, based on numerous experiments carried out with careful temperature control, are sufficiently valid to make it appear certain that the present method is not suitable for all types of egg products, such as yolks, whites and various mixtures.

The summary of their work on egg ash and various fixatives follows:

Egg white yields an ash free from carbon on ignition at 500°C. when magnesium acetate is added; egg yolk does not.

The minimum weight on the magnesium acetate solution, used as a fixative of the phosphorus in eggs, is not obtained on ignition at 500°C., even after prolonged heating.

When magnesium acetate is added as a fixative agent, it requires a temperature of 650°–700°C. to produce an ash on egg yolk free from carbon or to yield a minimum weight on the magnesium acetate.

There is a gradual loss of ash constituents in egg white when ignited at 650°–700°C., with or without the addition of magnesium acetate.

Calcium carbonate, in the presence of organic matter, undergoes little or no change on ignition at 500°C.; it decomposes gradually though irregularly and reaches minimum weight on ignition at 700°C.; and it does not undergo further change on ignition at 900°C.

Magnesium carbonate, in the presence of organic matter, undergoes decomposition but does not reach uniform weight on ignition at 500°C.; it reaches minimum weight on ignition at 700°C.; and it does not undergo further change on ignition at 900°C.

Potassium carbonate, in the presence of organic matter, retards greatly the combustion of carbon on ignition at 500°C.; it volatilizes appreciably on ignition at 700°C.; and it volatilizes almost completely on ignition at 900°C.°

Sodium carbonate, in the presence of organic matter, retards somewhat the combustion of carbon on ignition at 500°C.; it remains almost unchanged on ignition at 700°C.; and it volatilizes appreciably on ignition at 900°C.

A mixture of equal parts of potassium and sodium carbonates, in the presence of organic matter, behaves similarly to potassium carbonate on ignition, although the loss at 700°C. is distinctly less.

When potassium and sodium carbonates are ignited at 500°C. (thoroughly charred) in the presence of organic matter and magnesium acetate, and leached, there is apparently no loss. When these carbonates are ignited alone at 650°C., there is little or no loss.

When potassium and sodium carbonates are ignited at 700°C. in the presence of organic matter and magnesium sulfate, there is a large and variable loss of sulfur and there is no agreement between the weight of the magnesium salt in the blank and in the sample.

When magnesium acetate and sulfuric acid are added to eggs with a view to fixing both the phosphorus and the basic constituents, the lack of agreement in composition of the magnesium salt in the blank and in the yolk ash is particularly emphasized.

Ashing egg yolk in accordance with the official method general for food products,[1] using the leaching modification, yields an ash appreciably low in phosphorus.

The procedure of ashing egg material in the presence of magnesium acetate and

[1] *Methods of Analysis, A.O.A.C.*, 1925, 116, par. 7.

of determining the water-soluble and water-insoluble portions separately is unsatisfactory because of the uncertainty in composition of the magnesium salt.

The procedure of ashing egg material in the presence of a large excess of magnesium acetate is unsatisfactory, because the changes which the magnesium acetate undergoes on ignition in the blank are different from or are not simultaneous to the changes it undergoes in the sample.

CONCLUSION

Procedures of charring or ashing, followed by no or repeated leaching, with or without fixatives, have consistently failed to yield concordant results on different trials. At present there is no satisfactory method available for the determination of ash in eggs.

It is apparent that the tentative method does not give an ash that retains all the inorganic constituents of the egg, particularly in the case of egg white. Whether it will be possible to devise a method that will determine all the inorganic constituents and be satisfactory for routine work is not known, but it is believed that concordant results for some of the ash constituents can be obtained.

Based on the above considerations, it is recommended that the tentative method for the determination of ash in eggs[1] be dropped and that an attempt be made to devise a method suitable for all types of eggs.

WATER-SOLUBLE PROTEIN-NITROGEN PRECIPITABLE BY 40 PER CENT ALCOHOL

The egg constituent that is sought in this method is albumin, and the title "Albumin Nitrogen" is shorter and more significant than the above ponderous one. It is therefore recommended that the title "Albumin Nitrogen" be substituted for the present one.

The work of last year was continued by the referee. The method of filtering and washing the alcohol precipitate was abandoned when it was found definitely that the alumina cream carried down with it varying amounts of albumin. The indirect method, with slight modification, proved quite satisfactory for determining the albumin in the prepared solution. There was no loss of accuracy and the time consumed by the analyst was less in this method than in the direct one. The indirect method has the great advantage of reliability, as the procedure does not give a chance for the precipitate to become colloidal.

The greatest difficulty has been encountered in the preparation of the albumin solution, particularly with fresh eggs. The tentative method has been found difficult of manipulation and unreliable. Plimmer[2] states: ". . . on adding about a half a volume of ether to a solution of albumin and mixing thoroughly by inverting the liquids, a gelatinous solution results which contains coagulated protein." Both Mitchell and the referee have found that the ether precipitates varying amounts of albumin, de-

[1] *This Journal*, 12, 55 (1929).
[2] Practical Organic and Biochemistry. Longmans, Green and Co., London (1926).

pending in part on the time of contact. Plimmer states that the precipitate formed when an excess of alcohol is added to a dilute albumin solution is at first capable of re-solution in water, but on prolonged contact with alcohol it is rendered insoluble, the protein being coagulated. Methods have been described by Hardy and Gardiner[1] for obtaining serum proteins by precipitation with cold alcohol (below 0°C.) and resolution in water. This method was found inapplicable to egg white, since precipitation with absolute alcohol at −8° to 0°C. caused coagulation of most of the albumin.

The referee attempted to render the extraction of fat with ether less troublesome by removing the water in the egg sample with small proportion of alcohol. Ethyl ether containing 2 cc. of alcohol per 100 cc. was found to give concordant comparative results on egg whites, yolks and mixtures of known composition.

Some attempts at direct extraction of the albumin with water were made, but they were not successful, particularly with whole eggs, because of the difficulties involved in filtering. Mitchell was more successful, and obtained direct aqueous extracts by adjusting the acidity before filtering. He filtered the solutions of whites and yolks directly, and adjusted the acidity of known mixtures of whole egg until theoretical results were obtained. The change-point of cresol red was found to be the most satisfactory end point. The filtrations with egg yolk are tedious, and the referee has found the adjustment of acidity difficult, but the chances for coagulation are practically eliminated. Mitchell has been able to obtain higher and more consistent results than the referee's modification yielded. Mitchell also used the indirect method of determining albumin nitrogen recommended by the referee.

Table 2 gives the results obtained by ten collaborators on Mitchell's modification of the albumin nitrogen method. The maximum variation on the first sample sent out, No. 7573, is 0.21 per cent, and the average deviation from the average is 0.05 per cent, or 6 per cent of the average quantity of albumin found. The maximum variation on No. 7469 is 0.23 per cent and the average deviation 0.04 per cent, or 5 per cent of the average quantity of albumin. The agreement in results is too poor for the purposes of this determination in eggs.

Mitchell's results on No. 7573 indicated that the albumin nitrogen decreased on freezing, or some part of it was rendered unprecipitable by alcohol, since the albumin nitrogen decreased by 0.09 per cent, whereas the total water-soluble nitrogen decreased by only 0.03 per cent. It may be noted, however, that one of the collaborators obtained higher results on the frozen sample than Mitchell did on the fresh sample. The average results on No. 7649, which was prepared under aseptic conditions and

[1] *Proc. Physiol. Soc., J. Physiol.*, **40**, 68 (1910).

sent out in the liquid state, are 0.03 per cent higher than those for No. 7573, which was sent out in a frozen condition. This small difference might be due to the difference in the composition of the two samples. However, Mitchell's results on No. 7649 indicate that liquid eggs kept at room temperature even under as nearly sterile conditions as could be managed show a steady decrease in alcohol-precipitable nitrogen.

The results on the dried egg yolk sample are unsatisfactory. The referee suggested a modification which rendered the filtration of the aqueous solution simpler, quicker, and more certain. Results obtained by this method were practically identical with those obtained by filtering directly through filter paper. No comparative results are yet available as between the direct aqueous extraction methods used in this collaborative work and the methods involving previous extraction of the fat, which have been used heretofore.

The referee made some preliminary studies of the use of trichloracetic acid as an albumin precipitant. It has the advantage that the precipitate may be filtered at once and washed completely in a few minutes, and the nitrogen may then be determined directly. At the present time it seems that the acid also precipitates some globulin or pseudo-globulin, and therefore does not give so sharp a distinction between whole eggs and yolks as is desirable. It may prove worth while to remove the globulin by saturation with sodium chloride and to precipitate the albumin with trichloracetic acid.

In spite of the time and effort spent on this problem, it cannot be said that a satisfactory method has yet been evolved. The albumin-nitrogen value is of sufficient importance to justify continued attempts to work out a satisfactory method. It is recommended that the study of this method be continued, that comparison be made of the direct water extraction and the preliminary ether extraction, and that the use of trichloracetic acid as an albumin precipitant be further investigated.

The determination of albumin nitrogen by difference has been found much safer than the present direct method. In order to avoid mechanical coagulation of albumin it is advisable to specify gentle mixing instead of vigorous shaking. It is therefore recommended that the method for the determination of albumin nitrogen[1] be amended as follows:

ALBUMIN NITROGEN

PREPARATION OF SAMPLE

Powdered egg.—Place approximately 2 grams of sample into an 8-ounce nursing bottle, add 25 cc. of ethyl ether, stopper with a water-soaked cork, mix thoroughly but gently for several minutes, centrifugalize until the supernatant liquid is clear, and carefully decant off the ether solution, allowing none of the egg to be carried along. Re-extract three times with 20 cc. portions of ether in the same manner. Dry the fat-free residue by aid of suction and reduce to a fine powder by work-

[1] *This Journal*, 12, 56 (1929)

ing with a glass rod. Add 100 cc. of water from a pipet, mix well to avoid lumping, and add exactly 100 cc. more of water. Mix the contents of the stoppered bottle gently on a slowly revolving wheel or by hand for one hour. The temperature of the water should not exceed 30°C.

DETERMINATION

Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Determine nitrogen in 50 cc. of the filtrate by the official method,[1] making certain that the free flame does not touch the flask above the level of the liquid. Heat with a moderate flame for 1 hour after clearing. Distil into 20 cc. of 0.1 *N* acid. Run a blank on the reagents.

Pipet off 100 cc. of the above filtrate into a 200 cc. flask, add 15 cc. of sodium chloride solution (28 grams made up to 300 cc.), fill almost to the mark with ethyl alcohol, mix, cool to room temperature, make up to volume with alcohol, mix well and allow to stand overnight. Pipet off the supernatant liquid and filter through an 18½ cm. fluted filter paper. Determine nitrogen in 100 cc. of the filtrate as above. (To avoid bumping, add glass beads or delay the addition of potassium sulfate and mercuric oxide until the alcohol has been boiled off.) Subtract the value obtained from the water-soluble nitrogen to obtain the albumin nitrogen.

The direction for reagents and preparation of sample for liquid eggs from the following details of the method used by L. C. Mitchell should replace the present tentative method for liquid eggs. For the convenience of next year's Associate Referee on Albumin Nitrogen Mitchell's details for dried eggs are included here.

REAGENTS

(a) *Sodium chloride solution.*—Dissolve 28 grams of sodium chloride in water and dilute to 300 cc.

(b) *Cresol red indicator.*—Grind 0.1 gram of the dry powder in an agate mortar with 5.3 cc. of 0.05 *N* sodium hydroxide solution and when solution is complete, dilute to 25 cc. with water. Dilute 5 cc. of the stock solution to 50 cc. with water.

PREPARATION OF SOLUTION

(c) *Liquid eggs.*—Weigh accurately, by difference, approximately 10 grams of the well-mixed sample into a 250 cc. volumetric flask containing 150 cc. of water and mix gently. For whites or for yolks, fill to the mark with water, shake gently, and filter through an 18½ cm. fluted filter paper. If the filtrate is cloudy, allow to filter until drops of filtrate become clear, return the cloudy filtrate to the filter, and wash the receiving container twice with clear filtrate, returning the washings to the filter. For whole eggs or for mixed whites and yolks, add 5 cc. of 0.01 *N* acetic acid solution, mix gently, remove 1 drop of the egg solution to a spot plate containing 2 drops of water, and add 1 drop of cresol red indicator. Repeat the addition of 5 cc. of acetic acid solution, mixing and testing with the indicator on the spot plate until the coloration disappears. Fill to the mark with water, shake gently, and filter through an 18½ cm. fluted filter paper, returning the filtrate to the filter if it is cloudy.

(d) *Dried eggs.*—Transfer approximately 5 grams of sample to a 250 cc. volumetric flask containing 150 cc. of water and mix gently. Allow to stand for 1 hour with frequent gentle shaking so as to avoid the occurrence of foam. Filter through an 18½ cm. fluted filter paper, returning the filtrate to the filter if it is cloudy.

[1] *Methods of Analysis, A.O.A.C.*, 1925, 8.

DETERMINATION

The determination is carried out essentially as described in the method above.

To overcome the difficulties involved in the above procedure for dried eggs, the referee suggested the following:

To the sample in an 8-ounce nursing bottle add 200 cc. of water from a pipet, swirling continuously during the addition of the first 50 cc. Mix gently and allow to stand for 1 hour, mixing thoroughly every 10 minutes. Centrifugalize for 10 minutes and filter through a piece of cotton packed quite loosely in the stem of a 10 cm. funnel. (This should run through rapidly). Filter this filtrate through an 18½ cm. fluted filter paper and proceed as above.

The total time for centrifugalizing and filtering by this procedure was 45 minutes, whereas Mitchell's procedure on the same sample required 3 hours for filtering.

DETECTION OF DECOMPOSITION

Associate Referee H. D. Grigsby submitted a report on acid-soluble phosphoric acid. A sample of dried egg yolk was sent out for collaborative analysis by two methods which had appeared promising when preliminary work was done with them in the associate referee's laboratory. Concordant results were not obtained by either method, and the associate referee concludes that it is impossible to explain the discrepancies without further work.

The method previously used involved determining the phosphorus in a sulfuric acid solution by the volumetric method. The writer found that the presence of such an amount of sulfuric acid caused high results, even when the precipitation was carried out at 28°–30°C. with stirring.[1] Both of the procedures tried out this year avoid this error, one by fuming off the sulfuric acid, the other by making a dry ashing with potassium hydroxide. It is interesting to note that none of the methods proposed during the past few years employ the gravimetric method for phosphoric acid used by Pine[2] in gathering his valuable data on decomposition in eggs. It appears possible that the efforts to avoid the use of the gravimetric method may involve more difficulties than the recognized advantages of the volumetric method warrant.

The extraction of the phosphoric acid seems to offer the greatest chances of error. It is stated in MacLean's authoritative work[3] that picric acid slowly decomposes lecithin in the cold. It is known also that strong acids will decompose lecithin under certain conditions. Since picric and trichloracetic acids are both highly ionized, it would seem desirable to substitute a protein precipitant which is less active. Trichloracetic acid

[1] *This Journal*, 12, 170 (1929).
[2] *Ibid*, 8, 57 (1924).
[3] Lecithin and Allied Compounds—The Lipins, Longmans, Green & Co., London (1918).

will not precipitate all the water-soluble nitrogenous substances in egg, and it is probable that some of this material contains phosphorus.

The associate referee recommends that further work be done to perfect one of the two methods for acid-soluble phosphoric acid; that work on total bacteria count initiated in his laboratory be continued; and that further study be made of dried egg albumin to develop methods for detecting decomposition in this product.

The referee indorses these recommendations but wishes to urge that preference be given to work on ammonia nitrogen. A method for this determination,[1] which is the best chemical index of decomposition in frozen eggs, has been in use in food laboratories for a number of years. It should not require a great deal of effort to get this method adopted by the association.

TOTAL PHOSPHORUS (P_2O_5), FAT, LIPOIDS, AND LIPOID PHOSPHORIC ACID (P_2O_5)

Associate Referee J. H. Bornmann submitted a report covering the work of Clarke and Mitchell on these methods and his comments thereon. The method for fat by acid hydrolysis did not yield satisfactory results. This is attributed to insufficient hydrolysis. The referee notes that the directions in this work called for the use of concentrated hydrochloric acid in both liquid and dried eggs, whereas all previous work on eggs and cereal products has involved the use of more dilute acid for dried products, and concentrated acid only in the case of liquid eggs. The associate referee suggests that hydrolysis at a higher temperature may yield more concordant results.

No work was done on the present tentative method for lipoids. Instead, the associate referee reports results on a method involving the use of a chloroform-alcohol solution in the cold. The method has failed to give satisfactory results. Whether this is the fault of the method, or whether it is due to sudden and unexplainable decomposition in the eggs, is not clear at this time. A sample of liquid whole egg was analyzed by the writer and found to contain 12.68 per cent of lipoids, and 0.37 per cent lipoid P_2O_5 was placed in the refrigerator at 33°–39°C. overnight. The next day another analyst found 11.8 per cent of lipoids and 0.11 per cent of lipoid P_2O_5. The sample appeared as fresh as it had the previous day, and no apparent separation had occurred. In a private communication Mitchell describes an experiment with other portions of the sample. Several samples were allowed to become putrid, but showed no appreciable change in lipoids or P_2O_5, either on standing at room temperature or in storage in a refrigerator for a number of days. On the other hand, one sample which was allowed to stand for 16 days showed a lipoid content of 11.9 per cent, about 0.8 per cent lower than the others, and a lipoid P_2O_5 content of 0.02 per cent.

[1] U. S Dept. Agr. Bur. Chem. Bull., 846 (1920).

The method for total phosphorus (P_2O_5) reported on by the associate referee is patterned after the association method for chlorine in fruit ash. It has yielded excellent collaborative results.

The associate referee recommends that the method for total phosphorus given in his report be adopted as tentative, and be studied collaboratively in comparison with the present tentative method.

The referee suggests that a special study be made by the associate referee of methods for lipoids and lipoid P_2O_5 before submitting them to collaborative comparative study.

TOTAL NITROGEN

This method was not recommended for special study this year. However, it is listed by the Revision Committee as official (first action), and it should go into the new book of methods as an official method. Based on the data presented in Table 3, furnished by Clarke and Mitchell, this method is recommended for adoption as official (final action).

NEW STUDIES

The following methods are desirable ones for future study by the association: (1) chlorine; (2) glycerol, both qualitative and quantitative; (3) sterols; and (4) methods to detect decomposition in dried eggs.

Chlorine

(1) The chlorine determination is of value at present chiefly for the detection of added salt. Clarke and Mitchell obtained satisfactory results (see Table 4) for chlorine in whole eggs and dried egg yolk, but have not yet attempted to determine added salt. It is conceivable that the method, which is based on the association's method for chlorine in fruit ash, may give trouble due to decrepitation if large amounts of salt are present. Ulex[1] has described a simple method of determining added salt by precipitation of the protein and direct titration of the salt in the filtrate. It is recommended that the method described below[2] be adopted as tentative for the determination of chlorine in eggs and egg products, and that comparative study be made of this method and that of Ulex for determining added salt in eggs

Glycerol

(2) Glycerol, like salt, is added to liquid egg yolk as a preservative. Since it is added in sufficient quantities to affect materially the analytical constants on eggs, it is considered more feasible at this time for the referee on eggs to study this method than to refer it to the Referee on Preservatives. A rapid qualitative test for glycerol would be of value for routine examination of imported egg yolk

[1] *Chem. Ztg.*, 51, 758 (1927); *C.A.*, 22, 121 (1928).
[2] This method has been published in *Methods of Analysis*, *A.O.A.C.*, 1930.

Sterols

(3) Lampert[1] obtained promising results in the use of the cholesterol value as a criterion of the egg content of dairy products. The method seems to be adapted to other products. The irregularities found in the lipoid P_2O_5, heretofore considered the most reliable index of the egg content of food products, make it desirable to develop data on other, more stable, constants. It may be that a method for sterols will give more satisfactory results than the one for unsaponifiable matter. Recent investigations by Eikichi Igarashi[2] and Kenzo Kusui[3] indicate that the relation between cholesterol and total unsaponifiable matter in an unfertilized incubated egg changes with the period of incubation time. The method may therefore prove of value in judging decomposition.

(4) The associate referee on decomposition in eggs recommended that work on total bacteria count initiated in his laboratory be continued. There are no reliable methods available as yet for the detection of decomposition in dried egg albumin. It is suggested that attention be given to the acidity of the albumin and to the degradation products of the proteins.

REVISION

In consideration of the revision of *Methods of Analysis*, the following suggestions have been made to the Revision Committee:

COLLECTION AND PREPARATION OF SAMPLE

Par. 2, Liquid Eggs.—The directions to examine for foreign material are irrelevant. The last sentence should read: "Note odor and appearance."

Par. 3, Frozen Eggs.—What little evidence is available on segregation of moisture and solids indicates that there may be a marked settling of solids to the bottom of the can, so that the borings should extend *all* the way to the bottom. From certain theoretical considerations it seems advisable to take the borings at the center of the mass, which is on a circumference two-thirds of the distance from the center to the outer circumference of the can. The paragraph should therefore be modified as follows:

(b) *Frozen Eggs.*—Secure representative container or containers. Examine the contents as to odor and appearance. The condition of the contents can be determined best by boring a hole to the center of a container with an auger and noting the odor as soon as the auger is withdrawn. If it is impracticable to secure individual containers, samples may consist of the composite of the borings made on the contents of each container. Borings should be taken at points two-thirds of the distance from the center to the circumference of the can, from at least three widely separated parts and should extend all the way to the bottom of the can. Collect about 300 grams of the sample. Keep hermetically sealed in a jar in a cool place and in a frozen state if possible. Before analysis warm the samples in a bath held below 50°C. and mix well without removing the lid, but do not agitate violently.

Par. 4, Powdered Dried Eggs.—There appears to be no good reason why the general rules of sampling, which provide that the sample should be representative of

[1] *Ind. Eng. Chem.*, Anal. Ed., **2**, 159 (1930).
[2] *C. A.*, **23**, 1654 (1929).
[3] *Z. Physiol. Chem.*, **181**, 101 (1929); *C.A.*, **23**, 3256 (1929).

the outer portion of the package as well as the interior, should not be followed here: The following change is therefore recommended:

Par. 4, line 2.—For boxes and barrels, withdraw cores from a representative number of containers by means of a flour- or other suitable trier. Place the combined sample totaling about 300–500 grams in an hermetically sealed jar. Note the odor and appearance. Prepare the sample for analysis by passing it through a 20-mesh sieve and mixing thoroughly. Keep in an hermetically sealed jar.

Par. 5, line 2.—For the sentence "Report odor and appearance and examine for foreign material," substitute the following: "Note the odor and appearance."

Organic and Ammoniacal Nitrogen

Par. 13, lines 2 and 3.—Omit "500 cc., or preferably 800 cc."

Par. 13, line 5.—Change the first sentence to read: "Determine the nitrogen as directed on p. 8, 24."

The Kjeldahl-Gunning-Arnold modification is generally regarded as the best of the three official methods, and it is undoubtedly the best for this particular product. Furthermore, it is being recommended for the other nitrogen methods in eggs.

Fat (Acid Hydrolysis Method)

Direct hydrolysis in the Mojonnier tube has been adopted for flour and alimentary pastes and proved a decided improvement when applied to eggs in this year's work. The details of the tentative method should therefore be amended slightly, as follows:

Omit Preparation of Solution.

Par. 17, Liquid Eggs.—Weigh accurately by difference approximately 5 grams of the sample into a Mojonnier fat extraction tube. Add 10 cc. of hydrochloric acid, mix well, set the tube in a beaker containing water held at 75–80°C., and shake at frequent intervals for 30–40 minutes. Add sufficient alcohol to bring the level of the liquid within approximately 5 mm. of the top of the lower bulb and cool. Add 25 cc. of ethyl ether . . . , etc. Continue as in 19, line 3, to the end of the paragraph.

Par. 18, Powdered dried eggs.—Weigh accurately approximately 2 grams of the sample into a Mojonnier fat extraction tube, add 2 cc. of ethyl alcohol, and shake to moisten all particles. Add 10 cc. of hydrochloric acid (25+13), mix well, and continue as for liquid eggs, Par. 17.

RECOMMENDATIONS[1]

On the basis of work done during the year, it is recommended—

(1) That the method for the determination of total phosphorus described in the associate referee's report be adopted as tentative and be further studied collaboratively, with a view to its adoption as an official method.

(2) That the method for the determination of fat (by acid hydrolysis) described in the associate referee's report be studied in comparison with the present tentative method, and the more satisfactory method be studied collaboratively.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 59 (1931).

TABLE 1.

Results for total solids in whole eggs and dried yolk.

ANALYST	7573 WHOLE EGG (FROZEN)	7649 WHOLE EGG (LIQUID)	7648 EGG YOLK (DRIED)
	per cent	*per cent*	*per cent*
Mitchell	25.23[1]	25.23	96.11
Chicago	25.24[1]	25.24	96.11
	25.14	25.23	
	25.15	25.25	
		25.20	
		−25.21	
Clifford	25.27		
Washington	25.30		
	25.30		
Stone	25.06	25.47	96.00
Minneapolis	25.07	25.50	96.01
	25.09		
Aumer	25.30	25.26	95.96
St. Louis	25.31	25.25	95.95
Alfend		25.28	96.03
St. Louis		25.25	96.03
Horst	25.23	25.34	95.20[2]
New Orleans	25.28	25.38	95.30[2]
	25.29		
McCarthy	25.18	25.23	96.11
Cincinnati	25.21	25.24	96 04
Reed		25.33	95.88
Seattle		25.33	95.89
Average	25.21	25.29	96.01
Maximum	25.31	25.50	96.11
Minimum	25.06	25.20	95.88
Range	0.25	0.30	0.23
Av. Dev. from Av.	0.08	0.07	0.06

[1] Analyzed before freezing.
[2] Not included in average, range, etc.

TABLE 2.

Results for nitrogen in whole eggs and dried yolk.

ANALYST	NITROGEN					
	WATER-SOLUBLE			ALBUMIN		
	7573 WHOLE EGG (FROZEN)	7649 WHOLE EGG (LIQUID)	7648 EGG YOLK (DRIED)	7673 WHOLE EGG (FROZEN)	7649 WHOLE EGG (LIQUID)	7648 EGG YOLK (DRIED)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Mitchell	1.20[1]	1.23[2]	1.16	0.90[1]	0.94[2]	0.50
	1.20[1]	1.21[2]	1.18	0.89[1]	0.92[2]	0.51
	1.20[1]	1.21[3]		0.90[1]	0.89[3]	
	1.18	1.22[4]		0.82	0.88[4]	
	1.15	1.21[4]		0.79	0.87[4]	
	1.17	1.14[5]		0.82	0.83[5]	
		1.15[5]				
Clifford	1.15	1.21	1.23	0.76	0.83	0.59
	1.16	1.24	1.26	0.78	0.84	0.59
Stone	1.15	1.04	1.12	0.76	0.71	0.47
	1.19	1.14	1.20	0.81	0.79	0.49
	1.21			0.83		
Aumer	1.10	1.17	1.10	0.75	0.86	0.48
	1.15	1.15	1.10	0.80	0.83	0.47
Alfend	—	1.16	1.13	—	0.79	0.47
		1.32	1.13		0.95	0.47
Horst	1.14	1.19	1.25	0.89	0.86	0.77
	1.16	1.20	1.37	0.90	0.87	0.82
	1.24			0.96		
McCarthy	1.21	1.29	1.32	0.76	0.84	0.70
	1.16	1.29	1.27	0.78	0.89	0.54
Reed	—	1.11	1.02	—	0.83	0.33
		1.10	1.02		0.82	0.32
Salinger	—	1.19	1.16	—	0.89	0.54
San Francisco		1.20	1.16		0.88	0.53
Macomber	—	1.16	—	—	0.89	—
Savannah		1.17			0.90	
Average	1.17	1.19	1.18	0.83	0.86	0.53
Maximum	1.24	1.29	1.37	0.96	0.94	0.82
Minimum	1.10	1.04	1.02	0.75	0.71	0.32
Range	0.14	0.25	0.35	0.21	0.23	0.50
Av. Dev. from Av.	0.03	0.05	0.07	0.05	0.04	0.09

[1] Analyzed before freezing.
[2] Broken out Sept. 10; analysis started five hours later.
[3] Broken out Sept. 10; analyzed Sept. 11; 29 hours at room temp.
[4] Broken out Sept. 10; analyzed Sept. 12; 53 hours at room temp.
[5] Broken out Sept. 10; analyzed Sept. 26; 16 days at room temp.; had spoiled by Sept. 12.

TABLE 3.

Results for total nitrogen in whole eggs and dried yolk.

ANALYST	7573 WHOLE EGG (FROZEN)	7649 WHOLE EGG (LIQUID)	7648 EGG YOLK (DRIED)
	per cent	*per cent*	*per cent*
Mitchell	1.97[1]	2.02[2]	5.10
	1.96[1]	2.00[2]	5.08
	1.98	2.00[3]	
	1.98	2.03[3]	
		2.01[4]	
		2.00[4]	
Clifford	1.98	2.02	5.10
	1.97	1.99	5.07
Stone	1.99	1.89	5.16
	1.99	2.03	5.09
	2.03		
Aumer	1.96	1.95	5.06
	1.97	2.01	5.04
Alfend	—	1.97	5.02
		2.00	5.05
Horst	2.02	2.04	5.08
	2.02	2.04	5.16
	2.02		
McCarthy	1.95	2.07	5.13
	1.90	2.04	—
Reed	—	2.04	5.18
		2.04	5 16
Salinger	—	2 01	5.09
		2.01	5.08
Macomber	—	1.96	5.01
		2.00	5.05
Average	1.98	2.01	5.09
Maximum	2.03	2.07	5.18
Minimum	1.90	1.89	5.01
Range	0.13	0.18	0.17
Av. Dev. from Av.	0.02	0.03	0.04

[1] Analyzed before freezing
[2] Broken out Sept. 10; analysis started 5 hours later.
[3] Broken out Sept. 10; analyzed Sept. 11; 29 hours at room temp.
[4] Broken out Sept. 10; analyzed Sept. 12; 53 hours at room temp.

TABLE 4.

Collaborative results on chlorine and sugars in liquid eggs and dried yolk.

ANALYST	7648 DRIED EGG YOLK	7649 WHOLE EGG (LIQUID)	7573 WHOLE EGG (FROZEN)	
	CHLORINE (NaCl)		REDUCING SUGARS AS DEXTROSE	SUCROSE
	per cent	*per cent*	*per cent*	*per cent*
Mitchell	0.54	0.27	0.33	none
	0.53	0.27	0.33	
McCarthy	0.58	0.28	0.20	0.02
	0.55	0.27	0.30	none
Aumer	0.56	0.28	0.31	0.01
	0.56	0.28	0.31	none
Alfend	0.54	0.30		
	0.55	0.28		
Reed	0.47	0.26		
	0.47	0.25		
Horst	0.54	0.28	0.28	none
	0.54	0.28	0.29	
Stone	0.54	0.27	0.30	0.02
	0.55	0.28	0.30	0.03
Macomber	0.54	0.25		
	0.52	0.27		
Clifford	0.55	0.27	0.32	none
	0.55	0.28	0.31	
Salinger	0.59	0.29		
	0.57	0.29		
Average	0.54	0.28	0.31	
Maximum	0.59	0.30	0.33	
Minimum	0.47	0.25	0.20	
Range	0.12	0.05	0.13	
Av. Dev. from Av.	0.02	0.01	0.02	

(3) That special studies be made by the associate referee on the method for the determination of lipoids and lipoid phosphoric acid described in the associate referee's report, in comparison with the present tentative methods, and that the more satisfactory method be subjected to collaborative study.

(4) That Mitchell's method for the determination of sugar, described in this report, be studied with a view to its adoption as a tentative method

for reducing sugars and for added sugars. This study should include the collaborative analysis of egg samples containing known amounts of added sugars.

(5) That the 98°C. vacuum-oven method for total solids, with the slight modifications of detail described in the referee's report, be adopted as official (final action).

(6) That the tentative 112°–117°C. air-oven method for total solids be dropped, and work on this method be discontinued.

(7) That the tentative method for ash in eggs be dropped.

(8) That an attempt be made to devise a method for ash applicable to all types of eggs.

(9) That study of the method for unsaponifiable matter be continued.

(10) That the title of the method "Water-Soluble Protein-Nitrogen Precipitable by 40 per cent Alcohol" be changed to "Albumin Nitrogen"; that the method be modified according to the details described in the referee's report; and that this method be further studied in conjunction with the same methods for alimentary pastes and flour.

(11) That further work be done to perfect the method for acid-soluble phosphoric acid.

(12) That the present official (first action) method for organic and ammoniacal nitrogen, with the slight changes described in the referee's report, be made official (final action).

In consideration of the need for checking over the methods for inclusion in the new edition of *Methods of Analysis*, it is recommended—

(13) That the changes described in the referee's report for Methods of Sampling and Preparation of Sample be adopted.

(14) That the slight changes in the tentative method for the determination of fat (by acid hydrolysis) be adopted

For new studies, it is recommended—

(15) That the method for the determination of chlorine described in this report, with supportive collaborative data, be adopted as tentative, and that a comparative study be made of this method and that of Ulex for the determination of added salt.

(16) That a study be made of methods for the detection and determination of glycerol.

(17) That a study be made of methods for the determination of sterols.

(18) That a study be made of total bacteria count as an index of decomposition.

(19) That a study be made of methods for detection of decomposition in dried egg albumin.

In continuation of old recommendations, it is recommended—

(20) That prompt attention be given to a study of methods for determining ammonia nitrogen as an index of decomposition in liquid eggs.

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REPORT ON FAT BY ACID HYDROLYSIS, LIPOIDS, LIPOID PHOSPHORIC ACID (P_2O_5), AND TOTAL PHOSPHORIC ACID (P_2O_5) IN EGGS

By J. H. BORNMANN (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

This report is based on the work done by Clarke and Mitchell[1] in their investigation on eggs, and it includes the pertinent results obtained on collaborative samples sent out by them.

The following methods of analysis were used:

PREPARATION OF SAMPLE

(a) *Shell Eggs.*—Separation of Whites and Yolks. Chill eggs overnight in refrigerator. Break and carefully separate whites and yolks, collecting them in different containers. Mix whites by means of a malted milk stirrer just sufficiently to break cellular structure but without producing any foam, then stir thoroughly with a spatula or a spoon. Mix yolks thoroughly by means of a malted milk stirrer. Keep samples in a cold place. Report gross weight of eggs, average weight per egg, weights and percentages of whites, yolks, shells, and loss (if any), and, on the edible portion, the percentages of whites and yolks.

(b) *Liquid Eggs.*

(c) *Frozen Eggs.*

(d) *Dried Eggs.*

(e) *Flaked and Drum-dried Eggs.*

Follow the method laid down in *This Journal*, 9, 56 (1926).

Notes.—In breaking and separating eggs, yolks may become broken and accidentally intermingled with the whites. Such eggs should be discarded from the sample. To avoid loss of sample, place eggs, one dozen at a time, in the pan of a rough balance (E. & A. No. 17042, suitable type), note total weight, and weight after removal of each egg. Break egg on knife edge and carefully separate the white from the yolk by means of a commercial egg (yolk) separator, transferring the separated white and yolk to different, clean and dry, weighed, fruit jars. Mix the sample thoroughly by means of a spatula or a spoon each time before removing portions for analysis.

For analysis, portions of the prepared sample are weighed accurately by difference, the Bailey graduated weighing buret (E. & A. No. 19002) being used. The buret may be filled conveniently by inserting the lower end into the sample, then applying gentle suction by mouth to a heavy-walled rubber tubing of suitable diameter, 5–6 inches long or longer, fitted over top of buret. After filling the buret the lower end is wiped clean. Weigh out as rapidly as possible all portions needed for analysis, since on standing the egg material may separate within the weighing buret. If a Bailey weighing buret is not available, a small sized flask equipped with a rubber stopper, pipet, and a small rubber bulb in a manner similar to a dropping bottle, is a satisfactory substitute.

[1] Unpublished.

CHLORINE AND PHOSPHORUS

REAGENTS

(a) *Sodium carbonate solution.*—Dissolve 10 grams of sodium carbonate in water and dilute to 100 cc.

(b) *Olive oil.*

PREPARATION OF SOLUTION

(c) From the well-mixed sample, weigh accurately, by difference, approximately 4 grams of yolk, or 7 grams of whole eggs, or 10 grams of whites into a 250 cc. low-form Pyrex beaker; add 20 cc. of the sodium carbonate solution and evaporate to dryness on an electric hot plate or overnight at 100–105°C. Add 1 cc. of olive oil to the whites. Transfer the beaker while hot to an electric muffle heated to 500°C. (faint redness) and allow it to remain at this temperature for 1 hour. Cool, add a few drops of water, break up the charge with a glass rod, and cover the beaker with a watch-glass. Add 50 cc. of water, then add slowly and with continuous stirring 10 cc. of nitric acid (1+3) and filter, collecting the filtrate in a 250 cc. volumetric flask. Wash the charred material and filter thoroughly with water from a wash-bottle, fill to mark with water, and mix.

DETERMINATION

(e) *Phosphorus.*—Transfer 100 cc. of the prepared solution to a 300 cc. Erlenmeyer flask and determine phosphoric acid (P_2O_5) as directed on p. 3, 10, *Methods of Analysis*, 1925, using 35–45 cc. of molybdate solution. Report as total P_2O_5.

NOTE.—Ignition with sodium carbonate is official for chlorine (*Methods of Analysis*, 1925, p. 43, 11). Tilden[1] shows minimum quantity of sodium carbonate required. Repeated tests for both chlorine and phosphates, after igniting the washed charred residue to an ash, have been negative. This method, ashing with excess magnesium acetate, and wet digestion yield closely agreeing results for total phosphorus (P_2O_5).

FAT (ACID HYDROLYSIS)

PREPARATION OF SOLUTION

(a) From the well-mixed sample, weigh accurately, by difference, approximately 1 gram of yolks, or 3 grams of whole eggs, or 5 grams of whites into a Mojonnier fat extraction tube, add slowly 10 cc. of strong hydrochloric acid with vigorous shaking, set the tube into a water bath held at 70°–75°C., and shake vigorously at frequent intervals until the sample forms a smooth mixture free from coagulated protein particles. Add sufficient 95 per cent alcohol nearly to fill the lower bulb of the tube, and cool.

DETERMINATION

(b) To the extraction tube add 25 cc. of ethyl ether, and mix well. Add 25 cc. of redistilled petroleum ether (b. p. below 60°C.), and mix well. Let stand until the ether layer is clear. Decant as much as possible of the clear ether-fat solution into a weighed 125 cc. beaker-flask, dry it in an oven at the temperature of boiling water and then allow it to stand in the air to minimum weight. Re-extract the liquid remaining in the tube twice, each time with only 15 cc. of each ether. Shake well on the addition of each ether, allow to stand until the ether layer is clear, and decant the clear ether into the 125 cc. beaker-flask. Evaporate the ethers slowly on a steam bath, and dry in a boiling water oven to minimum weight (approximately 90 minutes). Remove the fat-flask from the oven, allow it to stand in the air until no further change in weight takes place (30 minutes), and weigh. Correct this weight by a blank determination on the reagents used. Report as "Fat by Acid Hydroly-

[1] *This Journal*, 11, 200 (1928).

NOTE.—While a few minutes at 70°C., or a much longer time at room temperature, is ample to hydrolyze the egg proteins, the treatment, however, does not appear to be sufficiently vigorous to assure complete splitting off of the phosphorus.

LIPOIDS AND LIPOID PHOSPHORIC ACID (P_2O_5)

REAGENTS

(a) *Mixed solvent.*—Equal volumes of chloroform and absolute alcohol.

(b) *Alcoholic potassium hydroxide solution.*—Dissolve 40 grams of potassium hydroxide in 1 liter of 95 per cent alcohol.

PREPARATION OF SOLUTION

(c) Weigh accurately, by difference, approximately 2 grams of the well-mixed sample into a 100 cc. volumetric flask, add very slowly (drop by drop) from a pipet with constant shaking until the proteins become coagulated and then thoroughly broken up, 25 cc. of the mixed solvent, add 60–65 cc. more of the solvent, and allow to stand 1 hour, mixing at 5 minute intervals. Fill to mark with the solvent, shake thoroughly, and pour as rapidly as possible onto an 18½ cm. folded filter, covering the funnel with a watch-glass during filtration, and collecting the filtrate in a 200 cc. Erlenmeyer flask.

DETERMINATION

(d) Transfer 50 cc. of the prepared filtrate to a 150 cc. beaker, and evaporate the extract to just dryness on a steam bath (an electric fan, or a gentle blast of dry air, may be used to hasten evaporation). Place beaker into a boiling water oven for 5 minutes to remove any remaining moisture. Dissolve the dry extract in 10 cc. of chloroform, and filter the solution into a weighed flat-bottomed platinum dish through a pledget of cotton packed into the stem of the funnel. Transfer through filter into a platinum dish by means of chloroform from a wash-bottle all soluble extract from beaker bottom and sides. Finally wash funnel and stem tip. (The filtrate should be clear.) Evaporate the chloroform on a steam bath, dry dish and contents in boiling water oven to minimum weight (approximately 90 minutes), cool, weigh and report the extract as percentage of lipoids.

LIPOID PHOSPHORIC ACID (P_2O_5)

PREPARATION OF SOLUTION

(e) Dissolve the dried lipoids obtained under (d) in 5 cc. of chloroform, add 10 cc. of the alcoholic potassium hydroxide solution, evaporate to dryness on a steam bath, and ignite in an electric muffle at 500°C. (faint redness) for 30 minutes. Remove dish from muffle, cover with a watch-glass, add 5 cc. of nitric acid (1+3), and filter. Wash the charred material and filter thoroughly with water from a wash bottle.

DETERMINATION

(f) In the filtrate prepared under (e), determine the phosphoric acid (P_2O_5) as directed on p. 3, 10, *Methods of Analysis*, 1925, using 20–25 cc. of molybdate solution. Report the P_2O_5 as percentage of lipoid phosphoric acid (P_2O_5) and as percentage of phosphoric acid (P_2O_5) in the lipoids.

NOTE.—As the solvent dissolves the egg moisture and water-soluble non-coagulable material as well as the lipoids, it is necessary to evaporate the extracted material to dryness and to dissolve the dry extract in chloroform in order to eliminate any water-soluble non-lipoid matter.

The first collaborative sample sent out, Inv. 7573, consisted of 108 infertile, one-day-old eggs obtained from a flock of pure bred single comb

white Leghorns. These eggs were carefully broken out August 11, 1930, thoroughly mixed, and analyzed August 12, 1930. Samples were placed in bottles in a sharp freezer on August 11, 1930, where they were held for several days. The bottles were wrapped in paper, packed in mailing tubes, and sent to the collaborators by mail August 18, 1930. The one exception was the sample sent to Washington, which was frozen for two days and mailed August 13, 1930. This sample arrived in good order, whereas all of those mailed August 18, 1930, except the New Orleans sample, were received broken. The collaborators reported as follows:

San Francisco.—Bottle was broken in transit, some of the contents had leaked out. Could not state whether there was any segregation of contents. Amount salvaged was equal to about one-half of the bottle.

Savannah.—The bottom of the bottle was cracked all the way around when received and broke out as I was transferring the contents to another bottle.

Cincinnati.—On arrival at this laboratory bottle showed a crack entire length of bottle (vertical) and around edge of bottom. Egg was solid, therefore none of sample had been lost. Bottle was immediately placed in beaker where, upon thawing out, the bottom of the bottle fell out. The contents were caught in the beaker and transferred to a pint fruit jar. In view of the fact that the sample had not completely come to room temperature at time of breakage, some moisture may have condensed on the egg during the time of transfer from the beaker to jar.

The sample had been well packed for shipment and package arrived here in good condition. Judging by the appearance of the cracked bottle, the possibility of the crack being caused by extreme temperature or expansion of the egg in freezing occurred to me.

St. Louis.—Container was found cracked when received. Parts of the bottle held together firmly so that none of the sample leaked through to the paper wrapper. It had to be rapidly transferred to another bottle, causing some loss. About one fourth of the sample was still frozen.

Minneapolis.—Jar was received with the bottom cracked but probably the portion of the sample analyzed was not affected by this breakage since the material in the neighborhood of the break was removed before analysis.

Some of the collaborators did not state the date on which analysis was started, but it is presumed that the work was started promptly on receipt of the sample. The results are given in Table 1.

After the results on the first collaborative sample had been received it was decided that some minor modifications should be introduced into the methods for future work. The changes were as follows:

PHOSPHORIC ACID

REAGENTS

(a) *Sodium carbonate solution.*—Dissolve 10 grams of sodium carbonate in water and dilute to 100 cc.

(b) *Olive oil.*

PREPARATION OF SOLUTION

(c) From the well-mixed sample, weigh accurately, by difference, approximately 2 grams of yolk, 4 grams of whole eggs, or 10 grams of whites into a 250 cc. low-form Pyrex beaker; add 20 cc. of the sodium carbonate solution; and evaporate to dry-

TABLE 1.

Inv. 7573, frozen whole egg—collaborative results.

ANALYST	FAT, ACID HYDROLYSIS	LIPOIDS	LIPOID P_2O_5	TOTAL P_2O_5
	FRESH—BEFORE FREEZING			
	per cent	*per cent*	*per cent*	*per cent*
Mitchell	11.06	12.74	0.37	0.55
Chicago	11.07	12.78	0.36	0.52
	ON FROZEN SAMPLE			
Mitchell	10.96	12.74	0.35	0.52
Chicago	11.05	12.72	0.36	0.54
Clifford	11.32	12.96	0.29	0.49
Washington	11.28	13.02	0.28	0.49
Stone	10.85	12.74	0.37	0.51
Minneapolis	10.93	12.83	0.39	0.52
Aumer	11.07	12.75	0.40	0.52
St. Louis	11.11	12.81	0.39	0.52
McCarthy	10.47	12.77	0.34	0.49
Cincinnati	10.43	12.71	0.35	0.49
Horst	11.16	12.75	0.35	0.55
New Orleans	11.01	12.58	0.37	0.55
Macomber	12.19	13.74	0.37	
Savannah	11.98	13.86	0.37	0.56
Salinger	12.2	18.8	0.38	0.55
San Francisco	12.2	13.9	0.37	0.54

ness on an electric hot plate or overnight at 100°–105°C. Add 0.5 cc. of olive oil to the whites. Transfer the beaker while hot to an electric muffle heated to 500°C. (faint redness) and allow it to remain at this temperature for 1 hour. Cool, add a few drops of water, break up the charge with a glass rod (flattened end), and cover the beaker with a watch-glass. Then add slowly and with continuous stirring 10 cc. of nitric acid (1+3) and filter, collecting the filtrate in a 300 cc. or 500 cc. Erlenmeyer flask. Thoroughly wash the charred material and filter with water from a wash-bottle.

DETERMINATION

(d) In the filtrate prepared as directed determine the phosphorus (P_2O_5), as directed on p. 3, 10, *Methods of Analysis*, 1925, using 40–50 cc. of the molybdate solution. Report as total P_2O_5.

The original method for fat by acid hydrolysis directed that the flask used in the final weighing be cooled in air. Modify this method to provide for cooling in a desiccator. On lipoids increase the weight of sample

from 2 grams to approximately 4 grams, and at the end of the determination cool the platinum dish in a desiccator. In the determination of lipoid phosphoric acid increase the amount of molybdate solution to 30–35 cc.

In examining the sample of dried yolk, weigh out samples according to the following schedule:

	grams
Phosphorus	1
Fat	1
Lipoids and Lipoid P_2O_5	2

Two additional samples were sent out for collaborative work. Inv. 7648 was a commercial spray-dried yolk, origin unknown. Results on this sample are given in Table 2.

TABLE 2.

Inv. 7648, spray-dried egg yolk—collaborative results.

ANALYST	FAT, ACID HYDROLYSIS	LIPOIDS	LIPOID P_2O_5	TOTAL P_2O_5
	per cent	*per cent*	*per cent*	*per cent*
Mitchell	60.06	64.05	1.74	2.62
	60.08	64.25	1.73	2.61
McCarthy		66.11	1.67	
	58.26	65.82	1.64	2.49
Aumer	59.23	64.13	1.75	2.60
	59.24	64.09	1.75	2.61
Alfend	59.22	63.73	1.75	2.66
	59.31	63.71	1.77	2.66
Reed	58.03	64.65	1.67	2.53
	57.91	64.60	1.67	2.53
Horst	59.23	64.17	1.95	2.60
	59.36	64.41	1.95	2.63
Stone	59.05	63.85	1.72	2.64
	59.37	64.11	1.72	2.64
Salinger	59.34	65.13	1.76	2.58
	59.29	64.95	1.76	2.59

The third collaborative sample, Inv. 7649, consisted of 9 dozen infertile eggs produced September 9, 1930, by the same flock of Leghorn hens as the first sample. These eggs were broken out September 10, under aseptic conditions, a dozen to a batch. Adhering white was removed with a policeman. The liquid was transferred to a quart fruit jar and mixed by means of an electric stirrer. After mixing, each one dozen batch was

poured through a 40-mesh sieve into a 1-gallon, wide-mouthed bottle; the entire sample was then mixed and poured into twenty 8-ounce wide-mouthed bottles. Ten samples were immediately mailed to collaborators; six were placed in a refrigerator as reserves; and the remaining four were left in the laboratory at room temperature. Analysis was started at Chicago, of one sample each on September 10th, 11th, and 12th, about 5

TABLE 3.

Inv. 7649, whole egg—collaborative results.

ANALYST	1930	FAT, ACID HYDROLYSIS	LIPOIDS	LIPOID P_2O_5	TOTAL P_2O_5
		per cent	*per cent*	*per cent*	*per cent*
Mitchell	9–10	11.12	12.52	0.37	0.51
		11.09	12.56	0.36	0.51
Mitchell	9–11	11.02	12.61	0.36	0.55
		11.07	12.62	0.37	0.54
Mitchell	9–12	11.04	12.60	0.38	0.51
		11.14	12.57	0.36	0.52
McCarthy	—	10.92	13.10	0.37	0.50
		10.93	12.99	0.37	0.51
Aumer	—	10.90	11.82	0.11	0.51
		11.15	11.84	0.11	0.51
Alfend	—	11.16	12.68	0.37	0.52
		11.17	12.68	0.37	0.51
Reed	—	11.02	12.55	0.31	0.49
		11.03	12.57	0.30	0.49
Horst	—	10.60	10.93	0.07	0.50
		10.73	11.09	0.08	0.52
Stone	9–12	11.18	11.59	0.08	0.50
		11.24	11.67	0.09	0.51
Salinger	9–12	10.98	12.64	0.33	0.50
		11.00	12.75	0.34	0.51

hours, 29 hours, and 53 hours, respectively, after breaking out the eggs. The fourth sample had spoiled by the third day. The collaborative results received at the date this was written are given in Table 3.

COMMENTS BY COLLABORATORS

Aumer.—Lipoids and lipoid phosphoric acid in the liquid whole egg are very low. A check on a 25 cc. aliquot on the same solution gave slightly higher results, possibly due to evaporation, although the bottles were stoppered. A check determina-

tion from the last remaining 10 grams of sample gave still lower results. Samples for this determination were taken after the egg had remained in the refrigerator (34°–39°F.) overnight. The platinum dishes were appreciably attacked by treatment of lipoid ash with nitric acid. Ammonium chloroplatinate separated out upon making the solution ammoniacal.

Alfend.—The two analysts (St. Louis) failed to check on lipoids, and particularly on phosphoric acid. The only difference in procedure was that Alfend's sample was weighed out the same day it was received, whereas Aumer's sample was weighed out after it had been in the refrigerator overnight, and the consistency seemed to have changed.

In dissolving the residue from the KOH fusion the dish was markedly corroded, the solution assuming the deep orange color characteristic of chloroplatinic acid. The precipitate of ammonium chloroplatinate which formed on neutralization presumably would have no effect on the P_2O_5 determinations. It is suggested that the directions be worded to specify removing all or most of the residue to a beaker before boiling with nitric acid solution.

The specified procedure involves a volume error which may be corrected for by the formula:

$$X = \frac{100(V-v)l}{Wa}, \text{ in which}$$

X = true percentage of lipoids,
l = wt. of lipoids found in aliquot,
a = volume of aliquot,
W = weight of sample,
V = total volume to which sample is made up, and
v = volume of insoluble solids in sample.

With the 2 gram sample of dried egg yolk v is approximately 0.75 cc. and X becomes 63.24 per cent. The determination is therefore about 0.5 per cent in error. With the 4 gram sample of liquid whole egg, v is approximately 0.5 cc. and X is 12.61 per cent. The error in this case is thus only 0.07 per cent.

Salinger.—In the fat determination the sample was transferred to Mojonnier tubes by aid of a small funnel. All egg was washed into the tube as completely as could be done, by use of the 10 cc. strong hydrochloric acid, together with the aid of a small wire. Any remaining substance that may have remained on the funnel was washed into the tube later by the alcohol and ethers.

The acid fat mixture was heated for about 5 minutes at the required temperature. The solution after this length of heating acquired a dark purple color. A preliminary experiment had shown this to be a satisfactory length of time, judging by the clearness and quickness of the separation of the ethers.

After constant weight was obtained, the fat was treated with petroleum ether. The result was corrected for by this residue, which amounted to 0.0006 gram. The ethers were not decanted till the solutions were very clear.

Blanks or controls were run on the lipoids and lipoid phosphoric acid. In the case of the lipoids, a correction of 0.0014 gram was subtracted from lipoids as weighed. On the lipoid phosphoric acid determination, the blank or correction was zero.

Macomber.—It seems to me that in the case of the fat determination, it would be well to emphasize the fact that the lower bulb of the tube is to be only partially filled with alcohol. The increase in volume after shaking with ether is just the opposite of the effect when extracting fat from other products such as cheese.

Clifford.—I suggest that in the preparation of the solution of lipoids the addition of a few lead shot to the 100 cc. volumetric flask after the volume has been completed to mark on shaking act to thoroughly break up the coagulated proteins.

DISCUSSION

The method used for the fat determination is the present tentative method with slight modifications. The agreement among collaborators is not quite satisfactory. This may be due to incomplete hydrolysis of lipoids. It is hoped that hydrolysis at a higher temperature will give more concordant results. The hydrolysis can be carried out in a Mojonnier tube to better advantage than in a beaker.

Most of the first samples sent out arrived with the bottles cracked or broken, probably caused by expansion in freezing. Mitchell found that samples held at room temperature for a week did not show material change in the constituents considered in this report. For this reason it was decided to send out the second sample of liquid egg without freezing. The samples for distant stations were sent by air mail; the sample for New Orleans was sent by special delivery; and the others were sent by parcel post. Samples analyzed by Mitchell and by Salinger on September 12th were apparently normal. The sample analyzed by Stone on September 12th and by Horst and Aumer, presumably on the same date, showed a marked decrease in lipoids and especially in lipoid phosphoric acid. It seems that lipoids may decompose quite rapidly in some cases, and this fact throws some doubt on the value of the lipoid determination in egg products.

There are several objections to the alcohol-chloroform extraction. One, as pointed out by Alfend, is due to the volume of the insoluble egg material; the other is due to a possible evaporation while filtering. Both would tend to give high results. It is hoped that these objections may be overcome, possibly by washing out completely instead of taking an aliquot. Some recent tests performed by Mitchell on washing the precipitated egg on a filter with the alcohol-chloroform mixture showed lower results on liquid egg and higher results on spray-dried yolk than were found by the procedure outlined in the methods. It appears that an alcohol-chloroform mixture is a better solvent for lipoids than alcohol and ether. Chloroform dissolves both fats and lipoids, whereas lipoids are sparingly soluble in ether.

Experiments carried out by Mitchell on ashing with sodium carbonate or with alcoholic potash gave some interesting results. It was found possible to do the ashing in Pyrex beakers, which will stand a temperature of 700°C., though at 800°C. they collapsed. With sodium carbonate the ash was gray at 500°C. or above, whereas, with potash it remained black even at 600°C., and, while it was gray at 700°C., it had fused at this temperature. The beakers showed much less etching with sodium carbonate. The filtrate was colorless in both cases with an ashing temperature of 500°C. The results on P_2O_5 recovered were excellent at all temperatures from 500°C. upward. This method should prove a great saver of platinum as well as time.

The results reported by collaborators on the total phosphoric acid determination are remarkably good. There was no adverse cirticism and it appears that this method is quite satisfactory as it now stands.

ACKNOWLEDGMENT

The writer wishes to acknowledge his indebtedness to J. O. Clarke and L. C. Mitchell, and to express his appreciation of the kindly manner in which the unpublished results of their investigation on eggs were made available for this report.

RECOMMENDATIONS[1]

It is recommended—

(1) That the modified method for the determination of total phosphorus given in this report be adopted as tentative and be studied collaboratively.

(2) That the modified method for the determination of fat by acid hydrolysis given in this report be studied collaboratively in comparison with the present tentative method.

(3) That the modified method for the determination of lipoids and lipoid phosphoric acid (P_2O_5) given in this report be studied collaboratively in comparison with the present tentative method.

For report of the Associate Referee on Water-Soluble Protein, Unsaponifiable Matter, Ash and Total Solids see the report of the General Referee on Eggs and Egg Products.

REPORT ON DETECTION OF DECOMPOSITION IN EGGS

By H. D. GRIGSBY (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

Two methods for the determination of acid-soluble phosphoric acid in dried yolk and dried whole egg were recommended, and one sample of dried yolk was sent out for collaborative work. In both of these methods the official volumetric method for P_2O_5 was used as the final step in the analysis.

On the basis of preliminary work done in the laboratory of the associate referee, these methods appeared promising, but the collaborative results were not satisfactory.

Method I (by B. B. Wright)

Weigh out 12 grams of commercial egg yolk, transfer to a 500 cc. centrifuge bottle, add 200 cc. of 1 per cent salt solution, shake until well mixed, add 10 cc. of hydrochloric acid solution (1+9) and 7 grams of picric acid, and mix on wheel or shaking machine for 30 minutes. Transfer to a 250 cc. flask, make up to mark, and shake; filter

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 59 (1931).

through dry filter and pipet 200 cc. of filtrate into a 500 cc. Kjeldahl flask. Add 10 cc. of concentrated sulfuric acid, a few glass beads, and 20 cc. of concentrated nitric acid. Boil gently until dense white fumes appear and then for 30 minutes longer. Cool, dilute with 25 cc. of water, and boil to dense white fumes again. Cool and wash contents of Kjeldahl flask into a 150 cc. low-type beaker, rinsing several times with small amounts of water and keeping the total volume to less than 80 cc. Place the beakers on the hot plate and let them go down to dryness slowly under the hood; cool, add a few drops of dilute nitric acid and 10 grams of ammonium nitrate, and determine P_2O_5 volumetrically.[1] Report as mg. of P_2O_5 per 100 grams of dried eggs.

Method II (by J. Fitelson)

Transfer 12 grams of dried egg yolk (24 grams of dried whole egg) to a centrifuge bottle, add 150 cc. of a 1 per cent salt solution containing 0.5 cc. of concentrated hydrochloric acid per 100 cc., and shake well. Add 6 grams of trichloracetic acid and again mix well. Place on a mixing machine for 30 minutes and centrifugalize until the supernatant liquid is almost clear. Decant into a 200 cc. graduated flask. Wash the residue twice with small portions of the NaCl-HCl solution, using the centrifuge. Dilute to 200 cc., mix, and filter through a dry paper. The filtrate should be clear.

Evaporate 150 cc.of the filtrate almost to dryness on a hot plate (using a platinum dish), add 10 cc. of 4 per cent alcoholic potash, and take down to dryness on a water bath. Ignite at a faint red heat until almost white. Extract ash first with hot water and decant through filter to remove chlorides, then acidify with nitric acid, keeping a watch-glass over the dish to prevent loss by spattering. Heat almost to

COLLABORATOR	METHOD I—BY B. B. WRIGHT	METHOD II—BY J. FITELSON
	mg. per 100 gram sample	mg. per 100 gram sample
H. I. Macomber	123.3 122.4	
I. A. Gaines	154.7 155.0	166.4 167.4
C. D. Schiffman	118.0 131.2	131.4 130.0
C. H. Hickey	160.3 157.4	134.6 133.6
L. C. Mitchell	139.0 132.0	164.0 167.0
S. Alfend	159.0 157.0	164.0 165.0
B. B. Wright	163.0 154.9	
J. Fitelson	157.7 159.0	152.4 150.9

[1] *Methods of Analysis, A.O A.C.*, 1925, 3, 10.

boiling, filter, and wash well with hot dilute nitric acid (1+9). Neutralize the filtrate with ammonia and determine P_2O_5 volumetrically.[1] Report as mg. P_2O_5 per 100 grams of dried egg.

DISCUSSION

The collaborative results obtained on acid-soluble P_2O_5 in dried egg yolk have been tabulated. All determinations were made on one sample.

The agreement among analysts for either method is not satisfactory, and it is impossible to explain the discrepancies without further work.

Some work has been done in the associate referee's laboratory on the total bacteria count in dried eggs in comparison with the acidity of the fat as an added indication of decomposition. These figures appear to be of some value, but they are not yet ready for publication.

Some preliminary work was done on dried egg albumin to find a point of attack for a chemical index of decomposition, but no results of value were developed.

RECOMMENDATIONS[1]

It is recommended—

(1) That further work be done to perfect one of the two methods for acid-soluble P_2O_5.

(2) That work on total bacteria count be continued.

(3) That further study be made of dried egg albumin to develop methods for detecting decomposition in this product.

No report on food preservatives was given.

REPORT ON COLORING MATTERS IN FOODS

By C. F. JABLONSKI (U. S. Food and Drug Administration, New York, N. Y.), *Referee*

In compliance with last year's recommendation of the association, the referee sent out four samples of mixtures containing tartrazine and amaranth to six collaborators, with a request to estimate the dyes quantitatively. The method submitted differed somewhat from the one suggested last year. Reports from five collaborators were received.

The composition of the mixtures (based upon titanium trichloride titration) was as follows:

	TARTRAZINE *per cent*	AMARANTH *per cent*	TOTAL COLOR *per cent*
1	88.22	8.32	96.54
2	8.82	83.19	92.01
3	92.43	4.36	96.79
4	4.62	87.15	91.77

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 59 (1931).

The results obtained by the collaborators are the following:

	TARTRAZINE per cent	AMARANTH per cent	TOTAL COLOR per cent
O. L. Evenson	84.20	14.20	98.40
Washington	12.00	79.90	91.90
	89.90	9.20	99.10
	2.70	89.40	97.37
D. B. Scott	90.80	6.57	97.37
New York	10.46	81.04	92.50
	94.16	3.64	97.80
	9.38	80.90	90.28
J. T. Bashour	88.00	8.98	96.98
New York	10.82	80.69	91.51
	93.90	3.00	96.90
	6.35	83.94	90.29
L. Jones	90.85	5.26	96.11
Kansas City	10.95	81.47	92.42
	92.18	4.64	96.82
	7.39	83.90	91.29
F. Hope	85.75	12.30	98.05
Kohnstamm &	9.10	84.75	93.85
Co., New York	86.60	11.40	98.00
	5.60	86.75	92.35

The following comments and criticisms were offered by the collaborators:

O. L. Evenson.—I suggest that sodium citrate be used as a buffer. This would obviate the use of a bitartrate factor. Sometimes a reddish purple color develops after centrifugalizing the first time. This was most pronounced in the case of No. 3. It seems that three portions of 0.25*N* hydrochloric acid might be sufficient.

D. B. Scott.—When a large amount of amaranth is present, I would suggest that a sixth funnel be used in order to get a more distinct separation of the orange and red colors.

J. T. Bashour.—In samples 2 and 4, containing comparatively large amounts of red, a noticeable quantity of red was washed out along with the orange in each case.

L. Jones.—I have no special comments or criticism to offer. I believe the results obtained are in fair agreement for a determination of this kind, since a difference of 0.1 cc. makes a difference of about 1 per cent in the final results. It is somewhat difficult to determine just when the orange II has been completely removed.

F. Hope.—The total dye appeared to be somewhat high and it occurred to me that the factor for the correction for amaranth titrated in the presence of sodium bitartrate was not correct for these small amounts of dye. The results of my investigation show that the factor for the correction of the titration of amaranth in the presence of sodium bitartrate is somewhat lower when one-tenth gram or less of dye is used.

Averaging the results obtained by collaborators, the following figures expressed in percentage are obtained:

	TARTRAZINE			AMARANTH			TOTAL COLOR		
	Reported	*Theory*	*Error*	*Reported*	*Theory*	*Error*	*Reported*	*Theory*	*Error*
(1)	87.92	88.22	−0.3	9.46	8.32	+1.1	97.38	96.54	+0 8
(2)	10.67	8.82	+1.9	81.57	83.19	−1.6	92.24	92.01	+0.2
(3)	91.35	92.43	−1.1	6.38	4.36	+2.0	97.72	96.79	+0.9
(4)	6.28	4.62	+1.7	84.98	87.15	−2.2	91.26	91.77	−0.5

From the reports given it will be noted that quantitative results may be obtained by the suggested method, as many of the results are within experimental error. It must also be stated that each 0.1 cc. of titanium trichloride titration represents approximately 1 per cent of dye, which accounts for discrepancies of some results. A phase of the problem that seemed to have caused considerable difficulty is the complete separation of orange II from fast red A. The referee therefore has taken cognizance of the suggestions offered and plans to embody them in the method to be submitted for final collaboration.

STUDY OF NEW DYES

A further recommendation of the association was for the referee to study the reactions and undertake separations of the last three adopted dyes (ponceau SX, sunset yellow FCF, and brilliant blue FCF) from the other permitted colors.

Ponceau SX

Ponceau SX dissolves in water, giving an orange red solution. Ammonia water or sodium hydroxide added to an aqueous solution of the dye produces an orange coloration. No appreciable change is noted upon the addition of a small amount of hydrochloric or sulfuric acid to the aqueous dye solution, but the addition of a large excess of either acid changes the solution to a brighter shade. Sodium hyposulfite ($NaHSO_2$) decolorizes readily a neutral solution of the dye, with more difficulty an alkaline solution, and only slightly an acid solution. Stannous chloride, titanium trichloride or zinc dust decolorizes ponceau SX. If to approximately 10 cc. of an aqueous solution 1 cc. of concentrated hydrochloric acid and 2 cc. of saturated bromine water are added, followed by excess of saturated hydrazine sulfate, and this mixture is coupled immediately with a solution containing two drops of alcoholic α-naphthol in 10 cc. of 2*N* sodium carbonate, a brownish red solution is obtained. Ether does not extract any color from this alkaline solution (differs from ponceau 3R). If to approximately 10 cc. of a neutral aqueous solution of ponceau SX a few drops of glacial acetic acid and 2 cc. of saturated bromine water are added, followed by an excess of saturated hydrazine sulfate solution, a brownish red color is produced (differs from ponceau 3R). Barium chlo-

ride or acetate does not produce any precipitate immediately in a neutral solution of ponceau SX, but after considerable time (several hours) a precipitate is formed, while a solution of neutral lead acetate does not produce a precipitate even after 24 hours (differs from ponceau 3R).

Spot tests on dyed wool:

Concentrated hydrochloric acid	—deeper red
Concentrated sulfuric acid	—brownish red
10% sodium hydroxide	—orange yellow
Strong ammonia water	—orange yellow

Ponceau SX is to a large extent extracted by amyl alcohol from a $N/16$ (approximately $0.06N$) hydrochloric acid solution, from which it may be removed by 5 per cent sodium chloride solution. Butyl alcohol extracts the coloring matter from a solution of the same acid concentration almost completely, from which it may be removed with water.

Dyes that may be extracted by solvents from the same acid concentrations and by their presence inhibit or distort the reactions, thereby giving rise to erroneous conclusions, are ponceau 3R and naphthol yellow S.

If to a neutral solution of ponceau 3R a small volume of 10 per cent barium chloride or acetate is added, a purple precipitate is obtained in a short time (less than 1 hour), and a brick red precipitate is formed upon addition of 20 per cent neutral lead acetate to a neutral solution of ponceau 3R (differs from ponceau SX). To establish further the presence of ponceau 3R, acidify approximately 10 cc. of the neutral solution with 1 cc. of concentrated hydrochloric acid, add 2 cc. of saturated bromine water and an excess of saturated hydrazine sulfate solution, and couple immediately with a solution containing 2 drops of alcoholic α-naphthol in 10 cc. of 2 N sodium carbonate. There is obtained a light orange coloration which dissolves in ether with a yellow color. This ether layer, after separation, is treated with a large excess of concentrated hydrochloric acid, when in the presence of ponceau 3R a purple coloration is produced (differs from ponceau SX). If to approximately 10 cc. of a neutral solution of ponceau 3R a few drops of glacial acetic acid and 2 cc. of saturated bromine water are added, followed by an excess of saturated hydrazine sulfate, the result will be a colorless solution (differs from ponceau SX).

To identify naphthol yellow S in mixtures with the ponceaus, the following procedure is advisable. A neutral solution of the mixed dyes is boiled with a few drops of 40 per cent stannous chloride solution, which will decolorize the azo colors, leaving the yellow practically unaltered in shade. After precipitation and removal of the tin by the addition of ammonia (avoiding excess) a yellow solution is obtained. This solution is turned orange with acids. As a confirmatory test the solution of the mixed dyes is treated with a small volume of a solution of barium chlo-

ride and allowed to stand overnight for complete precipitation. The precipitate of the ponceaus after filtering can be dissolved in amyl alcohol after acidifying and identified by the above methods. The naphthol yellow S solution is acidified and extracted with amyl alcohol, and the solvent is washed several times with small amounts of water. After the solvent has been diluted with gasoline the naphthol yellow S is removed with water. A characteristic test for naphthol yellow S is the following: The aqueous solution of the dye is made ammoniacal and a few crystals of sodium hyposulfite are added, when a red coloration is obtained in the presence of naphthol yellow S.

Sunset Yellow FCF

Sunset yellow FCF dissolves in water, giving an orange yellow solution. Strong ammonia water or a few drops of 10 per cent sodium hydroxide solution added to an aqueous solution of the dye gives a brownish red coloration. The addition of a small volume of concentrated hydrochloric acid produces very little change; a large excess of acid gives a more reddish color. Concentrated sulfuric acid added to a solution of sunset yellow makes it more pink. Sodium hyposulfite decolorizes readily a neutral or alkaline aqueous solution, and somewhat more difficultly a hydrochloric acid solution. Stannous chloride, titanium trichloride and zinc dust decolorize sunset yellow. To approximately 10 cc. of an aqueous solution, 1 cc. of concentrated hydrochloric acid and 2 cc. of saturated bromine water are added, followed by excess of saturated hydrazine sulfate, and this mixture is coupled immediately with a solution containing 2 drops of 1 per cent alcoholic α-naphthol in 10 cc. of $2N$ sodium carbonate. In the presence of sunset yellow a cherry red solution is obtained. (The above test is not specific for sunset yellow, as it is also obtained in the presence of orange I and tartrazine, in fact, wherever sulfanilic acid is the first component.)

Spot tests on dyed wool:

Concentrated hydrochloric acid	—slightly redder
Concentrated sulfuric acid	—slightly redder
10% sodium hydroxide	—browner
Strong ammonia water	—no change

Sunset yellow in $0.25N$ hydrochloric acid is almost completely extracted by amyl alcohol, from which it can be removed by 5 per cent salt solution. Butyl alcohol extracts a larger portion of the dye from $0.06N$ hydrochloric acid, from which it may be removed by water.

Dyes that may be extracted from the same acid concentration and by their presence inhibit or distort the reactions, thereby giving rise to erroneous conclusions, are indigotine, tartrazine and amaranth. To isolate and identify indigotine the neutral solution of the mixed dyes is treated

with small amounts of sodium hyposulfite ($NaHSO_2$), until the solution becomes colorless or nearly so. Upon the addition of acetic acid, and heating to expel sulfur dioxide, the indigo blue is restored, while the other dyes remain decomposed.

To a slightly ammoniacal solution of the mixed dyes a small volume of 3 per cent hydrogen peroxide is added; this treatment decolorizes and destroys the indigotine, leaving the sunset yellow FCF, tartrazine, and amaranth practically unaltered. Separation of sunset yellow from tartrazine and amaranth can be performed in the following manner:

Acidify the solution of these dyes with 1/10 volume of concentrated sulfuric acid and extract in 20 cc. portions with two 40-cc volumes of *n*-butyl alcohol. Reserve the lower layer for further treatment. Wash the butyl alchol extract successively with 20 cc. portions of a mixture containing 5 per cent anhydrous sodium sulfate in 0.2*N* sulfuric acid until the washings are colorless and add the washings to the reserved portion. Wash the butyl alcohol extract with water to remove the sunset yellow (its identity is confirmed by the wet and spot reactions mentioned above). The reserved portion may contain tartrazine or amaranth or both. By acidifying the solution strongly with hydrochloric acid and extracting with amyl alcohol, the coloring matter will be taken up by the solvent. Discard the lower layer and evaporate the extract carefully over a steam bath to dryness (avoid charring). Dissolve the residue in a small amount of water and divide into two portions. Make one portion of the solution alkaline with ammonia and carefully add a few crystals of sodium hyposulfite ($NaHSO_2$). Amaranth will be immediately decolorized, while tartrazine is attacked only with difficulty. Acidify this yellow solution and speedily extract with amyl alcohol, from which solvent the tartrazine can be removed with 0.25*N* hydrochloric acid and identified by the wet and spot reactions. The presence of amaranth can be established by acidifying with acetic acid the second portion of the aqueous solution and adding saturated bromine water and immediately a saturated solution of hydrazine sulfate. The presence of amaranth manifests itself by the formation of a pink solution, its intensity depending upon the amount of this coloring matter present; tartrazine treated similarly produces a colorless solution.

Brilliant Blue FCF

Brilliant blue FCF dissolves in water giving a blue solution. Ammonia or 10 per cent sodium hydroxide solution does not change the coloration in the cold. However, if heated to boiling in the presence of 10 per cent sodium hydroxide solution a purplish solution is obtained; this is converted to a blue shade by the addition of glacial acetic acid and an orange shade with mineral acid. Small amounts of concentrated hydrochloric or sulfuric acid added to a solution of brilliant blue FCF produce a greenish yellow and a strong excess of either acid a yellow solution. Sodium hyposulfite ($NaHSO_2$) decolorizes readily an alkaline or acid solution of the dye, but the color returns after standing and shaking with air; however, a neutral solution is not perceptibly affected by this reagent. If to approximately 10 cc. of an aqueous solution 1 cc. of sulfuric acid (1+4) and 2 cc. of 0.5*N* sodium nitrite are gradually added and the solution

is boiled vigorously to expel excess of nitrous acid a deep wine red color is formed which is changed to a slightly bluer shade by the addition of ammonia water and later to green after boiling (differs from light green SF yellowish and guinea green B). If to approximately 10 cc. of an aqueous solution 1 cc of glacial acetic acid and 2 cc. of saturated bromine water are added and then an excess of hydrazine sulfate, a blue coloration is obtained. This test is also given by fast green FCF (differs from light green SF yellowish and guinea green B).

Spot tests on wool:

Concentrated hydrochloric acid	—green, changing to yellow
Concentrated sulfuric acid	—green, changing to yellow
10% sodium hydroxide	—no change
Strong ammonia water	—no change

Brilliant blue is readily extracted by α-dichlorhydrin; it is also extracted to a large extent by n-butyl alcohol from a 0.25N hydrochloric acid solution, from which it may be removed by 5 per cent salt solution.

Dyes that may be extracted from the same acid concentration, and by their presence inhibit or distort the reactions, thereby giving rise to erroneous conclusions are: guinea green B, fast green FCF, and light green SF yellowish. Guinea green B is separated from the other triphenylmethane dyes by extraction from a 5 per cent salt solution with amyl alcohol.

Fast green FCF when treated with 1 cc. of sulfuric acid (1+4) and 2 cc. of sodium nitrite (as under brilliant blue FCF) produces a deep wine red coloration; the addition of ammonia water and boiling does not produce any change (differs from brilliant blue). If to 10 cc. of an aqueous solution of fast green FCF 1 cc. of sulfuric acid (1+4) is added, the solution boiled and gradually 2 cc. of 0.1N bromide-bromate solution are added and the excess of bromine boiled off, a blue coloration is produced. This coloration becomes redder by the addition of ammonia water and bluish violet upon the subsequent addition of acetic acid (differs from brilliant blue, light green SF yellowish and guinea green B). Light green SF yellowish and guinea green B, when treated with acid and sodium nitrite according to the method for brilliant blue FCF and fast green FCF, become light yellow or colorless; light green SF yellowish and guinea green B, when treated with acid and saturated bromine water (according to the method for brilliant blue FCF and fast green FCF) become light yellow or colorless.

To separate light green SF yellowish from fast green FCF and brilliant blue FCF, the following procedure is followed:

To the mixed dye solution add an equal volume of 2/N sodium carbonate and extract in 20 cc. portions with two or three 40-cc. portions of n-butyl alcohol. Reserve the lower layer for further treatment. Wash the butyl extract successively with 20 cc. portions of 0.5N sodium carbonate until no more color is extracted and

the upper solvent is practically colorless. Combine washings with the reserved solution. The butyl alcohol will contain the extracted light green SF yellowish, which may be removed from the solvent by the addition of a small volume of water and a few drops of acetic acid, a green solution resulting. The presence of this dye may be confirmed by wet and spot reactions.

To the aqueous solution of fast green FCF and brilliant blue FCF add an equal volume of 20 per cent anhydrous sodium sulfate (or approximately 10 grams of anhydrous sodium sulfate to every 100 cc. of solution). Then extract this solution in 20 cc. portions successively with two or three 40-cc. portions of *n*-butyl alcohol. Reserve the lower layer for further treatment. Wash the butyl alcohol with 20 cc. portions of 10 per cent anhydrous sodium sulfate solution until no more color is extracted, adding the washings to the reserved solution. In the butyl alcohol extract will be found the brilliant blue, which can be removed with water after the addition of gasoline and confirmed by the nitrite and bromine tests as well as spot tests. Acidify the remaining reserved solution strongly with concentrated hydrochloric acid and extract the fast green with a mixture consisting of 4 parts of benzyl alcohol and three parts of butyl alcohol. Remove the coloring matter from the solvent with water after the addition of gasoline and the fast green FCF is confirmed by the bromine, nitrite and bromide-bromate tests, as well as spot reactions.

Since the above methods are of a qualitative nature, the necessity of a quantitative method for separating the recently adopted dyes from other permitted coal tar dyes is of prime importance.

RECOMMENDATIONS[1]

The referee therefore recommends—

(1) That additional samples of mixtures of tartrazine and amaranth be submitted for collaborative work.

(2) That sample mixtures containing the recently adopted dyes in conjunction with other permitted colors be sent to collaborators in order to test the methods of separation and identification as herewith submitted.

(3) That work be undertaken to separate and estimate quantitatively the recently adopted dyes in the presence of other permitted colors.

REPORT ON METALS IN FOODS

By G. C. SPENCER (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

The work of the associate referees on metals in foods this year was marked by commendable activity. A large number of collaborators were solicited, and many responded with highly satisfactory results and with expressions of interest in the procedures that were under consideration. This should be especially gratifying to the association this year when the task of revising *Methods of Analysis* is before it.

ARSENIC

The practice of spraying fruits with arsenical preparations has made the methods for the estimation of arsenic of great importance in the regu-

[1] For report of Subcommittee C and action of the association see *This Journal*, 14 62 (1931).

latory laboratories. The present official Gutzeit method has been subjected to so many changes and modifications by chemists who have had wide experience in this line of work, that it was considered advisable to call a symposium for the purpose of revising the method and harmonizing the differences that have arisen. This symposium has already met, and the results of its deliberations have been expressed by a committee in the form of a new draft of the Gutzeit method based on the procedure presented by H. J. Wichmann. Because this method was incorporated in the 1930 revision of *Methods of Analysis*, it is not given here.

Chemists working on the determination of arsenic have long realized that the Gutzeit method should be supplemented, if possible, by a volumetric method that would be reliable for slightly larger quantities of arsenic than are provided for in the Gutzeit method. A volumetric procedure of this sort would obviate the errors that result from taking small aliquots.

The associate referee's report on arsenic for 1929[1] described a volumetric method for arsenic, known as the arsine distillation method. This year the bromate method has been added. These two procedures were referred to collaborative study this year with satisfactory results.

TIN AND COPPER

The simplified volumetric methods[2] which were proposed by the associate referees in 1929 were studied collaboratively this year. It is hoped that further work will establish the advantages of these methods over the present tentative procedures.

BORON[3]

Two points have been brought out by the associate referee's work this year. First, the possibility of more completely distilling off the boric acid from phosphoric acid with superheated steam. Bismuth salts are used in this reaction. Secondly, a note on the present official method,[4] showing that greater care must be observed to prevent the escape of boric acid with the vapors of methyl alcohol during distillation. No collaborative work was done on boron.

LEAD

The Associate Referee on Lead worked on a method that would be applicable to the determination of lead in spray residues. For this purpose the precipitation of lead as the chromate was tried with very satisfactory results. The recoveries of lead by this method from apple peelings, to which known weights of lead had been added, were so close that further study of this method is recommended for the coming year.

[1] *This Journal*, 13, 417 (1930).
[2] *Ibid.*, 423, 426.
[3] *Ibid.*, 422.
[4] *Methods of Analysis*, *A.O.A.C.*, 1925, 131.

RECOMMENDATIONS[1]

It is recommended—

(1) That the present official Gutzeit method for the determination of arsenic be dropped and that the revised Gutzeit method be adopted as an official method (final action).

(2) That the arsine distillation method for the determination of arsenic be further studied.

(3) That the bromate method for the determination of arsenic be further studied.

(4) That the volumetric method for the determination of tin presented by this year's associate referee be further studied.

(5) That the present tentative method for the determination of copper be dropped and the volumetric method presented by the present associate referee be adopted as a tentative method.

(6) That further study be made on methods for the estimation of boron.

(7) That further work be done on lead determinations.

REPORT ON ARSENIC

By W. C. TABER (Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

The associate referee sent out two solutions containing arsenic to about 30 collaborators who had expressed a willingness to cooperate. Reports were received from 21. Many of the chemists who reported had never tried either method submitted, and both of them demand some experience on the analyst's part before satisfactory results may be expected. The following letter was sent to all the collaborators. The procedure of Method I, called for convenience the arsine distillation method, has been published.[2] The bromate method is given in detail.

TO THE COLLABORATORS ON ARSENIC

The associate referee has thought it advisable to submit samples to try out two methods for the determination of arsenic that have recently been investigated. They both include distillation with subsequent titration. Method I, which may be called the arsine distillation into mercuric chloride, is based on the procedure suggested by C. R. Smith, Circ. 102, U. S. Bur. Chemistry (1912). The method is based on the fact that when arsine is passed into a mercuric chloride solution, arsenides and other arsenic and mercury compounds, which are titrated with an iodine solution, may be formed. Whatever compounds are formed the reaction with iodine is essentially a titration from AsH_3 to As_2O_5 according to the equation, $2AsH_3+16I+5H_2O=As_2O_5+16HI$. It is applicable for larger amounts of arsenic than can be accurately found by the Gutzeit method, and it is hoped that it may be applied directly to the wash solutions from fruit without any preliminary treatment. A preliminary report was made to the A.O.A.C. in 1929 in which good results were recorded, particularly when no organic matter is present.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 62 (1931).
[2] *This Journal*, 13, 418 (1930).

Method 2, called the bromate method, has recently been developed by H. R. Smith, now of the National Canners Research Laboratory, Washington, D. C. It is a modification of the official method for arsenic in insecticides. See Journal A.O.A.C., Vol. 5, pp. 33–50. It also has been adopted as a tentative method for drugs. It requires a preliminary digestion with acids before distillation. It involves a distillation of the arsenic as arsenious chloride in a current of hydrochloric acid gas and a subsequent titration with a standard potassium bromate solution.

Samples have been prepared without any organic matter, so no preliminary digestion is required. The samples are labeled No. 1 and No. 2, and contain about 130 cc. of solution each Use 25 cc. of each sample for a determination, and run in duplicate, 100 cc. therefore being required of each sample, and a remainder of 25 cc. being left for any emergency. In the bromate method no sulfuric acid is present, so it will be necessary to add 25–30 cc. of concentrated sulfuric to correspond to the amount that may remain in a sample after acid digestion. Blanks of course should be run on both methods. It is advisable to make preliminary runs in each method with known solutions in order to familiarize oneself with the procedure and to test the apparatus. This can be easily done with the standard arsenic solutions. Please report results as milligrams of As_2O_3 per 25 cc. of sample, and also as grams of As_2O_3 per lb., assuming that the 25 cc. represents 700 grams of fruit. . . .

NOTES

(1) The referee has used a standard arsenic solution of 0.4 gram of As_2O_3 per liter, slightly weaker than 0.01 *N*, and the iodine of approximately the same strength. When standardizing an iodine solution against a standard As_2O_3 solution the arsenic is oxidized by the iodine from the "ous" to the "ic" condition. However, when the iodine is used to oxidize the arsenic from the arsine to the arsenic oxide, four times as much iodine is consumed as in the former case. Therefore, determine the As_2O_3 equivalent of the iodine solution in the latter titration by dividing the As_2O_3 equivalent obtained in the former titration by 4. For example, the referee uses a standard arsenic solution containing 0.4 mg. of As_2O_3 per cc., and an equivalent iodine solution. This iodine solution, however, is equal to only $\frac{1}{4}$ of 0.4 mg. of As_2O_3, or 0.1 mg. of As_2O_3 per cc. when used in oxidizing arsine to As_2O_5.

(2) The amount of solution used in the unknown has been suggested as 400 cc. for the reason that the use of the wash solution from 700 grams of fruit has been tried directly in this method, and this amount of solution is necessary in the washing of the fruit.

Method II.—Bromate method

(As outlined by H. R. Smith)

In this method the procedure should start with No. 3, taking 25 cc. from the unknown solution and not making the preliminary digestion, and removal of nitric acid. It will be necessary, however, to add 25–30 cc. of concentrated sulfuric acid, and proceed as indicated.

REAGENTS

(a) *Concentrated nitric acid.*—Arsenic-free.
(b) *Concentrated sulfuric acid.*—Arsenic-free.
(c) *Concentrated hydrochloric acid.*—Arsenic-free.
(d) *Ammonium oxalate solution.*—5 per cent in H_2O.
(e) *Sodium or potassium bromide, c.p.*
(f) *Ferrous sulfate, c.p.*

(g) *Commercial salt (NaCl).*

(h) *Methyl orange indicator.*—0.5 gram per liter.

(i) *Standard potassium bromate solution.*—0.2813 gram per liter gives a solution (approx. 0.010 *N*) in which 1 ml. = 0.50 mg. of As_2O_3 (2).

(j) *Standard As_2O_3 solution.*—Dissolve 0.500 gram of As_2O_3 in dilute NaOH, make slightly acid with dilute H_2SO_4, and make up to 1 liter.

APPARATUS

One 800 ml. Kjeldahl flask for each determination.

One 300 ml. Erlenmeyer flask for each determination.

One distilling head. Bend a 10–15 mm. glass tube to an acute angle of about 60°. Fit the short arm, which is about 4 inches long, with a No. 7 rubber stopper (3). Draw down the end of the long arm, which is about 10 inches long, to a diameter of about 3 mm.

PROCEDURE

(1) *Acid digestion.*—As usual for the complete destruction of organic matter. Start with 30 ml. of H_2SO_4 and 50 ml. of HNO_3 (4).

(2) *Removal of HNO_3.*—To the acid residue add 50 ml. of H_2O, boil to white fumes, add 25 ml. of 5% $(NH_4)_2C_2O_4$, and boil again to white fumes.

(3) *Distillation of $AsCl_3$* (5). To the solution in the flask add 25 ml. of H_2O and *cool* to room temperature. Put 150 ml. of H_2O at room temperature into an Erlenmeyer flask and clamp into position (6). Have ready this mixture of salts: 0.5 gram of KBr (or NaBr), 2 grams of $FeSO_4+7H_2O$, and 30 grams of commercial NaCl, also 25 ml. of concentrated HCl in a cylinder. Add to the sample solution in the Kjeldahl flask the mixture of salts, then the concentrated HCl, and adjust the distilling head to distil from the Kjeldahl flask into the water in the Erlenmeyer flask. Heat the digestion mixture over a small well-protected flame (7). The absorption of evolved HCl gas by the water causes a rise in temperature, which furnishes an indication of the progress of the distillation. When the temperature of the water and acid solution has risen to 70°C., enough gas has been evolved to carry over all the $AsCl_3$ present. Adjust the flame so that the time of heating will be from 9 to 11 minutes. Lift off the Kjeldahl flask and the distilling head.

(4) *Titration of distilled $AsCl_3$.*—Heat the distillate nearly to boiling and titrate hot with the standard bromate solution, using 2 drops of the methyl orange indicator (8). Near the end the bromate must be added very slowly with constant shaking. Single drops of the indicator may be added during the titration if the color fades (9). The end point is an absolutely water-white solution when the last drop of bromate destroys the final tinge of red color. If the end point is overstepped, add small amounts of the standard arsenic solution and another drop of indicator. Blank determinations on the entire procedure should use not more than 0.40 ml. of the bromate solution (10). If difficulty is encountered in obtaining a satisfactory blank, test the HCl and distilled water for reducing substances by titrating 30 ml. of HCl and 150 ml. of H_2O with the methyl orange and bromate solution; 4 or 5 drops should be sufficient.

NOTES

1. This strength solution can be titrated to 1 drop. Using 1000 grams for a sample, 1.0 part of As_2O_3 per million gives 1.0 mg. of As_2O_3, which uses 2.0 ml. of this solution.

2. In checking the standard of the bromate solution always titrate with about 150 ml. of H_2O and 30 ml. of concentrated HCl in order to simulate the conditions under which samples will be titrated.

3. The rubber stopper used should be boiled with 10 per cent NaOH for 15 minutes, then boiled with concentrated HCl for 15 minutes in order to remove most of the sulfur compounds which might be distilled and react with the bromate.

4. It has been found that bumping during digestion is largely prevented by using only a *small* amount of HNO_3 at the beginning. The speed of digestion is largely determined by the rate of heating. Use an asbestos board with a 3″ hole.

5. All the arsenic present is reduced to $AsCl_3$ by the $FeSO_4$ through the KBr (HBr). The $H_2SO_4+HCl+NaCl$ evolves a large amount of HCl gas which carries the $AsCl_3$ over with it. The heating employed is not intended to boil the solution, but only to assist in the evolution of the HCl gas. The amount of HCl evolved in the heating prescribed was found to be 9.5–11.0 grams, which in gaseous form must have occupied a volume of about 7 liters.

6. Previous investigators have kept the distillate recciving flask in an ice bath. This has been found to be a needless precaution.

7. The flame is a Bunsen flame about 3″ high showing just a tinge of yellow at the top. A Fletcher chimney burner with an asbestos board having a 2″ hole is very satisfactory.

8. It should be remembered that the methyl orange indicator is not used as ordinarily to show color change from acid to alkali, but the red acid color of the methyl orange is oxidized by any excess of bromate to a colorless oxidation product. This reaction is not reversible under the conditions obtaining. The reaction takes a perceptible amount of time and near the end the bromate must be added very slowly. To establish the end point, compare the color with that of clear water in a similar flask. To show that the end point has not been *more* than reached, add 2 drops of the methyl orange indicator—the color should remain for 1 minute.

9. The use of more than 6 drops of the indicator before the end point should be avoided, since oxidation of the indicator consumes appreciable amounts of the bromate solution.

10. Correct the volume of bromate used for the blank determined, using all of the reagents and the regular distillation.

The arsenic solutions were prepared from the Bureau of Standards As_2O_3, according to the procedure advised by that Bureau in the preparation of the arsenic solution for titration purposes, viz: dissolving the arsenious oxide in pure sodium hydroxide and water and saturating this solution with carbon dioxide, thus converting all the sodium hydroxide to sodium bicarbonate. This solution was afterward oxidized by hydrogen peroxide to the arsenate form. Sample No. 1 contained 4 mg. of As_2O_3 per 25 cc., and Sample No. 2 contained 1.5 mg. of As_2O in the same volume.

The results obtained by the 21 collaborators are given in the table.

It will be noted that some results are entirely out of line with the theoretical and also with the recoveries secured by most of the analysts. It was thought that they should not be included in the averages.

Some comments of the collaborators are noted.

H. Heidenhain.—Arsine Method: I believe that the standard solutions are unnecessarily weak, and that the advantage of more accurate readings of the buret is offset by the uncertainty of titer. The merit of the method lies in the fact that it is based on absolute values and not, as is the Gutzeit method, on comparative values, which must be obtained under strictly uniform conditions, a thing not easily accomplished. *Bromate Method:* The error seems to be an absolute quantity de-

pending upon the quantities of chemicals, and also on the speed of distillation, but not upon the quantity of arsenic present.

M. J. Gnagy. Arsine Method: Extension Tube. Three different tubes were employed. The first had an inside diameter of about $\frac{3}{4}$ mm. A strip of mercuric bromide paper showed a loss of arsine in preliminary trials. The inside diameter of tube was approximately 0.5 mm. This gave smaller bubbles of gas and better contact of gas with solution. No appreciable loss was then noted. The greatest difficulty encountered was in the use of the iodine solution. Due to the handling of the iodine solution and to the time element in same, loss of iodine was constantly taking place. The ratio of the As_2O_3 solution to the iodine solution was constantly changing. The collaborator prefers the bromate method to the arsine distillation method because of the simpler operations, greater stability of solutions used, and shorter time to properly perform the operations.

The following results obtained by Gnagy on preliminary work on the arsene distillation method are given below:

DETERMINATION NO.	DATE WORK DONE 1930	As_2O_3 TAKEN	As_2O_3 RECOVERED	TIME OF OPERATION	REDUCING SOLN. USED	Zn USED
		mg.	*per cent*	*hour*	*cc.*	
1	6–11	4	97.37	1	50	Considerable left
2	6–11	4	96.48	1	50	Considerable left
5	6–13	4	99.83	2	50	Zn left
6	6–13	8	92.21	2	50	Zn left
7	6–14	4	100.32	2	100	Some Zn left
8	6–16	8	95.50	2	100	Some Zn left
9	6–17	8	94.55*	2¼	75	All Zn used
			1.17	1	25	
10	6–18	4	96.72*	2¼	100	All Zn used
			0.44	1		

* Two bottles used in series. The first figure represents percentage recovered in first bottle, second figure represents percentage recovered in second bottle and after first bottle was removed.

Dan Dahle.—Bromate Method: The main hardship of this method seems to be to bring the blank within reasonable limits. Even after using C.P.NaCl we were unable to bring the blank below 0.6 cc. *Arsine Method:* These tests seem to indicate that in its present state the method is apt to give slightly low results.

E. H. Berry.—Judging from my results the methods seem to be equally accurate. If it is eventually found that Method 1 cannot be adopted directly to the wash solution from the fruit, I believe that I should prefer Method 2.

J. Fitelson.—Arsine Method: Owing to the lower results secured by this method, Sample No. 2 was analyzed again as in the method, except that after 60 minutes of evolution the contents of the flask were brought to boiling. 1.59 mg. of As_2O_3 was found. Apparently it requires this final heating to drive over the last traces of arsine. If the arsine method could be made more accurate it would be preferable to the bromine method because of the sharper titration, ease of manipulation, and the fact that a number of determinations could be run at the same time. *Bromate Method:* Difficulty was experienced in securing a satisfactory blank. Replacing commercial salt with C.P. sodium chloride reduced the blank to 0.7 cc.

R. H. Robinson.—Arsine Method: I have been favorable to the arsine distillation method, having obtained excellent results on both known and unknown samples the last year. I did not, however, use the large amount of solution that would necessarily have to be used if the total washing were taken. When smaller volumes of the

unknown were taken, I have found the results to be fairly accurate. Whitaker, however, reports the method to be approximately 10 per cent low as indicated by results reported. The bromate method apparently gave promise. This method seems accurate, and furthermore is rapid and simple.

D. W. McLaren.—It is believed that in a write-up of the bromate method the errors caused by excess methyl orange should be stressed.

DISCUSSION

Some comparisons of the two methods may be given. In the bromate method a preliminary wet ashing is ordinarily necessary in order to destroy the organic matter. The subsequent distillation as arsenous chloride requires much less time than the arsine distillation. The latter, however, requires a much larger titration than that using the bromate solution, 1 cc. of the iodine being equivalent to 0.1 mg. of As_2O_3, while 1 cc. of the potassium bromate is equivalent to 0.5 mg. of As_2O_3. It must be conceded that at the present time it has not been possible to make good recoveries in using the acid or alkali wash of the fruit directly in the arsine distillation method, which was the hope of the associate referee reported last year. The discrepancies reported this year by some analysts on pure solutions has directed attention to this phase of the problem. The addition of potassium bromide as well as the iodide as a reducing agent was tried without success. The use of copper and iron as sulfate in very small amounts to speed up the reaction did not secure the additional 2–5 per cent necessary for complete recovery of the arsenic. The last procedure which we have just recently tried with good results differs from the method sent out in a few particulars. Instead of using the 100 cc. of reducing solution, its volume was decreased to about 60 cc. by using 50 cc. of concentrated hydrochloric acid. After the reaction with the zinc has continued for about one-half hour, heat the flask by means of an electric hot plate and add further amounts of tin to revivify the reaction. Two cc. of the stannous chloride solution is added to 100 cc. of concentrated hydrochloric acid, and this mixture is added gradually to the flask as usual. The associate referee has obtained recoveries in 5 known samples of 97.5, 98.4, 97.8, 98.1 and 98.6 per cent, while in the procedure outlined to the collaborators results have been obtained from about 94 to 97 per cent. In the bromate method the referee obtained in Solution 1 a recovery of 100 per cent, and in Solution 2, 102.9 and 96.3 per cent. Some points raised by the collaborators are worth further consideration. The use of the extension tube in the mercuric bromide solution is of importance. The referee emphasized the constriction to 1 mm. diameter at the lower end of the tube, and has found that sufficient with the depth of solution used in a one-half pint milk bottle, i.e. about 5 cm. If an Erlenmeyer flask were used for the bromide solution the depth of the liquid would not be sufficient. This necessary depth of solution should be emphasized. Analysts Dahle and Gnagy report some loss from the bromide solution. The former ar-

Results of collaborative work on arsenic in foods.

COLLABORATORS	METHOD NO 1						METHOD NO. 2					
	SAMPLE NO. 1			SAMPLE NO. 2			SAMPLE NO. 1			SAMPLE NO. 2		
	As_2O_3 PER 25 cc. (SOL. 1)	RECOVERY	ESTIMATED AS GRAINS PER LB †	As_2O_3 PER 25 cc (SOL 2)	RECOVERY	ESTIMATED AS GRAINS PER LB.	As_2O_3 PER 25 cc. (SOL. 1)	RECOVERY	ESTIMATED AS GRAINS PER LB.	As_2O_3 PER 25 cc (SOL. 2)	RECOVERY	ESTIMATED AS GRAINS PER LB.
	mg.	*per cent*		*mg.*	*per cent*		*mg.*	*per cent*		*mg.*	*per cent*	
J. P. Aumer	3.54	88.5	0.035	1.32	88.0	0.013	3.86	96.5	0.039	1.41	94.0	0.014
St. Louis	3.62	90.5	0.036	1.36	90.7	0.014	3.86	96.5	0.039	1.42	94 7	0.014
E. H. Berry	3.80	95.0	0.038	1.47	98.0	0.015	3 95	98.7	0.039	1.40	93.3	0.014
Chicago	3 82	95.5	0.038	1.45	96.7	0.014	3.85	96.2	0.038	1.40	93.3	0.014
H. F. Cope	3 65	91.2	0 036	1.426	95.1	0.014	4.20	105.0	0.042	1.66	110.6 *	0.016
Medford, Ore.	3.72	93.0	0 037	1.404	93.7	0.014	4.165	104.1	0.042	2.15	143.3	0.021
Dan Dahle	3.93	98.2	0.039	1.582	105.4	0.016	3.974	99.3	0.040	1.484	98.9	0.015
Savannah	3.72	93.0	0.038	1.568	104.5	0.016	4.059	101.5	0.041	1 559	103.9	0.016
J. E. Fahey	3.216	80.4	0.032	1.36	90.7	0.014	3.76	94.0	0.038	1.58	105.3	0.016
Wenatchee, Wash.	3.641	91.0	0.036	1.51	100.6	0.015	3.95	98.7	0.040	1.51	100.6	0.015
L. H. Feldstein	3.80	95.0	0.038	1.45	96.6	0.014	4.00	100.0	0.040	2.40	160.0 *	0.024
Denver	3.72	93.0	0.037	1.44	96.0	0.014	4.30	107.5	0.043	1.97	131.3	0.020
M. J. Gnagy,	3.82	95.5	0.038	1.40	93.3	0.014	4.08	102.0	0.041	1.48	98.7	0.015
Minneapolis	3.90	97.5	0.039	1.63	108.6	0.016	4.06	101.5	0.041	1.60	106.7	0.016
	3.84	96.0	0.038	1.45	96.7	0.015						
A. W. Hanson	3.88	97.0	0 039	1.40	93.3	0.014	4.05	101.2	0.041	1.52	101.3	0.015
Minneapolis	3.62	90 5	0 036	1.44	96.0	0.014	4.03	100.7	0.040	1.50	100.0	0.015
H. Heidenhain	3.83	95.7	0 038	1.53	102.0	0.015	4.60	115.0 *	0.046	1.90	126.6 *	0.019
Wenatchee, Wash.	3.82	95.5	0 038	1.46	97.3	0.015	4.30	107.5	0.043	2.10	140.0	0.021
P. P. Gray	3.63	90.7	0 036	1.36	90.7	0.014	4.04	101.0	0.040	1.85	123.3	0.018
New York City	3.70	92.5	0.037	1 38	92.0	0.014	4.01	100.2	0.040	1.49 1.62	99.3 108.0	0.015 0.016

Louis G. Petree	3.69	92 2	0.037	1.46	97.3	0.015	3.66	91.5	0.037	1.41	94.0	0.014
San Francisco	3.61	90.2	0.036	1.43	95.3	0.014	3.75	93.7	0.037	1.37	91.3	0.014
H. R. Smith, Wash, D. C.							4.00	100.0	0.040	1.50	100 9	0.015
William Seidenberg	2.61	65.2 *	0.026	0 59	39.3 *	0 006	4.133	103.3	0.041	1.793	119.5 *	0.018
New York City	2.66	66.5	0.027	0 95	63.3	0.009	4.208	105.2	0.042	1.793	119.5	0.018
Geo. Schumacher	3.828	95.7	0.038	1.328	88.5	0 013	3.70	92.5	0.037			
Medford, Ore.	3.725	93.1	0.037	1 328	88.5	0.013	3 60	90 0	0.036			
Frank A Vorhes	3 74	93 5	0 037	1 31	87.3	0 013	4.10	102.5	0.041	1.47	98.0	0.015
Seattle	3.61	90 2	0.036	1 25	83.3	0 012	4 05	101.2	0.040	1.47	98.0	0.015
A. S Mills				1 33	88.7	0 013	4.218	105.4	0.042	1.69	112.6 *	0.017
Portland				1 32	88.0	0 013	4.218	105.4	0.042	1.73	115.3	0.017
R. H Robinson	3 64	91.0	0.036	1.33	88.7	0.013	3.99	99.7	0.040	1.52	101.3	0.015
Ore. Agr. Coll.	3.64	91.0	0.036	1 39	92.7	0 014	4 09	102.2	0.041	1.56	104.0	0.016
J. Fitelson	2.89	72.2 *	0.029	1.29	86.0 *	0.013	4 45	111.2	0.044	1.41	94.0	0.014
New York City	3.22	80.5	0.032	1.11	74 0	0.011	3.91	97.7	0.039	1.39	92.7	0.014
Wm C. Woodfin	3 71	92.7	0.037	1.018	67.9	0.010	4.00	100.0	0.040	1.50	100.0	0.015
Baltimore	3.47	86 7	0.035	1.348	89.9	0.013						
	3.23	80.7	0.032	1.303	87.2	0.013	4 00	100.0	0.040	1.50	100.0	0.015
A. D. Rich	3.08	77 0	0.031	1.555	103.7	0.016	3.86	96.5	0.039	1.457	97.1	0.015
Chicago	3.865	96 6	0 039	1.355	90 3	0.014	3.96	99.0	0.034	1.508	100.5	0.015
	3.74	93.5	0.037	1.587	105.8	0.016						
	3.68	92 0	0.037	1.315	87.7	0.013						
D W. McLaren	3.182	79.5 *	0.032	1.485	99.0	0.015	4.12	103.0	0 041	1.57	104.7	0.016
Philadelphia	3.439	86.0	0 034	1.548	103 2	0.015	4.00	100.0	0.040	1.67	111 3	0.017
Average		92.0			94.0			100 1			102 9	

* Not included in average.

† 0 001 grain = 0 0648 mg

ranged the recovering bottle with a two-holed rubber stopper fitted with a glass tube for testing the escaping gas by the Gutzeit paper and found only a few micros of As_2O_3. The referee has tried the same procedure with similar results. The results given by Analyst Gnagy on his preliminary work seem to indicate a somewhat larger loss.

The difficulty of keeping a constant iodine solution of the strength named is referred to by a few of the collaborators. The referee has not found that trouble with ordinary precautions and a new titer at short intervals. The temperature of the locality may have something to do with this difficulty.

The chief trouble the associate referee had with the arsine distillation is in getting a complete evolution of the arsenic as arsine from the distillation flask. This has been proved in numerous trials of the residue in the distillation flask by running a Gutzeit on this solution. In most cases the amount found has accounted for the incomplete recovery. The suggestion of the referee of using more tin and additional heat seems the best procedure.

From the results of work done to date it appears that the arsine distillation method gives somewhat low results, and the bromate method high ones. In view of the experience in Western laboratories with the official Gutzeit method, in which the best results showed a plus or minus error of 10 per cent and even as high as plus or minus 20 per cent, it seems that these two methods merit consideration.

The associate referee suggests that the official method for arsenic in foods be modified to accord with the procedure now in force in the Western District of the Food and Drug Administration, a copy of which is being submitted by the District. One change is suggested, that of the impregnation of the paper by the bromide solution. The official method states that the paper should be soaked for one hour in the 5 per cent mercuric bromide solution. Analysts have universally followed this procedure. Experiments made by the associate referee, however, show that an immersion of the paper for 5 minutes is as good as an hour or more.

In reference to the rapid method for the determination of arsenic in apples and pears, the following data are submitted in justification of its use:

Analyses made by C. R. Gross, Seattle Station, by rapid method of using 3% HCl, but no preliminary treatment with chloroform

VARIETY	GRAINS PER LB. BY SOLVENT METHOD	Mg. As_2O_3 PER 10 CC. OF ALIQUOT OF HCl SOLUTION	DIGESTED PEELS	PERCENTAGE REMAINING IN PEELS
Spitzenberg	0.0201	55	3	8
Jonathan	0.0233	50	7	12
Not stated	0.0097	18	3	14
Spitzenberg	0.032	60	3	5

The following are some of the results obtained by Western Laboratories, Yakima, Wash., on different varieties of fruit run by the quick method involving a preliminary wash with chloroform and a subsequent wash with hot sodium hydroxide solution, as compared with the acid digestion method.

QUICK METHOD (grain per lb. As_2O_3)	DIGESTION (grain per lb. As_2O_3)
0.0050	0.0055
0.0120	0.0175
0.0040	0.0045
0.0110	0.0100
0.0250	0.0215
0.0120	0.0130
0.0244	0.0250
0.0060	0.0060
0.0045	0.0035
0.0040	0.0042
0.0100	0.0105
0.0055	0.0054
0.0150	0.0150
0.0220	0.0215
0.0175	0.0165
0.0315	0.0350
0.0230	0.0235
0.0052	0.0055
0.0265	0.0280
0.0045	0.0055
0.0052	0.0075
0.0055	0.0065

L. H. Chernoff, Denver Station, reported the following results obtained by the rapid method using alkali and acid:

Roman Beauty Apples

NaOH 3%	HCl 3%
0.008 grain As_2O_3 per lb.	0.0098 grain As_2O_3 per lb.
0.0006 grain As_2O_3 per lb. left on peelings	0.0015 grain As_2O_3 per lb. left on peelings
93% recovery	87% recovery

Winesaps

Found by rapid alkali wash	0.021 grain per lb.
Found left on peelings as determined by acid digestion	0.0009 grain per lb.
96% recovery	

Palmer and Gross, Seattle Station, report as follows on acid dip of fruit:

	GRAIN As_2O_3 PER LB.	RECOVERY *per cent*
Acid dip	0.0083	89.2
Digestion of peelings	0.0010	
Acid dip	0.0087	88.0
Digestion of peelings	0.0012	

J. P. Aumer, St. Louis, reports on 2 samples of apples by 3 per cent HCl washing, and subsequent wet ashing of the peel, and also of the HCL acid solution.

NO.	3% HCl WASHING	WET ASH OF PEELINGS AFTER HCl WASHING	WET ASHING OF HCl SOLUTION
1	0.011	0.001	0.011
check	0.011	0.001	0.011
2	0.019	0.002	0.019
check	0.019	0.002	0.019

W. J. McCarthy, Cincinnati Station, reports as follows on 6 samples of apples:

SAMPLE	RAPID ACID METHOD GRAIN As_2O_3 PER LB.	WET ASH OF PEELINGS AFTER WASHING	WET ASHING OF SOLUTION OF ACID WASHING
1	0.009	0.001	0.009
2	0.009	0.001	0.009
3	0.009	0.001	0.007
4	0.022	0.005	0.020
5	0.014	0.002	0.014
6	0.011	0.001	0.011

St. Louis Station reports results on various samples of apples, 1928 to 1930:

A	B	C	D	E
0.010	—	—	—	0.000
0.003	—	—	—	0.000
0.005	—	—	—	0.000
0.003	—	—	—	0.000
0.014	0.001	—	—	0.000
0.014	0.000	—	—	0.001
0.017	—	0.000	—	0.001
0.013	—	—	—	0.000
0.006	—	—	—	0.000
0.021	—	0.000	—	0.002
0.004	—	0.001	0.004	0.002
0.005	—	0.001	0.005	0.001
0.011	—	—	0.011	0.001
0.019	—	—	0.019*	0.002

A—Fruit washed in boiling 3% HCl solution.
B—Residual fruit from A washed in boiling 3% NaOH solution.
C—Residual fruit from A washed with chloroform, then washed in boiling 3% HCl solution.
D—Solution A, wet ashed.
E—Peel of residual fruit from A and/or B and/or C wet ashed.
* Entire sample.

RECOMMENDATIONS[1]

(1) In view of the coming conference on the Gutzeit method and the submission by the Western District of its procedure for following the Gutzeit method, no recommendation is made on this subject.

(2) Since the data submitted on the arsine distillation and bromate methods indicate that fairly accurate results may be secured on solutions

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 62 (1931).

without organic matter by analysts of some experience, it is recommended that these two methods be adopted as tentative.

SYMPOSIUM ON DETERMINATION OF ARSENIC

At the symposium on the determination of arsenic by the Gutzeit method held October 20, 1930, H. J. Wichmann of San Francisco presented some modifications of the official Gutzeit method for the determination of arsenic in food products based on analytical work done in the U. S. Food and Drug Administration. These modifications were discussed and incorporated in the revised method which was then adopted as official by the association. Wichmann also presented a graphic method of estimating the quantity of arsenic. In this method a curve is plotted, the quantities of arsenic and lengths of stains obtained from a series of standard stains being used as coordinates. By means of curves of this kind the quantity of arsenic in any particular sample can be readily found when the length of stain has been determined. The data and curves presented by Wichmann will not be reproduced here since the amount of arsenic should be determined only by a curve obtained from standard strips run at the same time and from the same strip-group.

REPORT ON BORON

By O. F. KRUMBOLTZ (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

Some time was devoted to a review of the present official method for distilling boric acid with methyl alcohol.[1] As stated in the associate referee's report in 1929,[2] a decided excess of alkali is necessary to absorb all the boric acid that distils over with the methyl alcohol, otherwise appreciable losses occur when the alcohol is distilled off.

It has also been found that when heated in glass containers alkaline solutions dissolve silicic and boric acids in sufficient quantities to affect the final titration. Owing to a balancing of errors the results are only slightly changed for larger amounts of boric acid, but with small amounts of boric acid the results are seriously affected.

At least three times the calculated quantity of alkali should be used for collecting the distillate, and the final alkaline solution should be evaporated to dryness in a platinum dish. The residue is dried and heated to about 250° for 30 minutes. It is then taken up in water and titrated as usual.

Recoveries by these modifications varied from 98 to 101 per cent.

Work was continued on the distillation method, superheated steam, which was first referred to in last year's report, being used.

[1] *Methods of Analysis, A.O.A.C.*, 1925, 18, par. 61.
[2] *This Journal*, 13, 422 (1930).

It was found that the presence of phosphoric acid seriously hindered the distillation of the boric acid owing probably to the formation of borophosphates. A study of the literature suggested that this difficulty might be overcome by the addition of bismuth salts, which would form stable compounds with the phosphoric acid and thereby liberate the boric acid. It was also found that when an excess of bismuth salts (bismuth subcarbonate) were present in the reaction mixture the results obtained were much better than when no bismuth was used.

Further study is needed to clear up this point. It is recommended that the work on analytical methods for the determination of boron be continued.

REPORT ON TIN

By ANNA E. MIX (Food and Drug Administration, Washington, D. C.), *Associate Referee*

The work on tin analysis for the past year was confined to the study of a method to determine its reliability in the hands of different analysts. This method is essentially the same as that recommended by the Associate Referee on Tin last year.[1]

Two unknown solutions were sent to each of 11 chemists, and reports were received from seven.

The aliquots recommended for analysis contained 23.74 mg. (Soln. A) and 4.75 mg. (Soln. B) of tin, respectively.

METHOD OF ANALYSIS

REAGENTS

(a) *Hydrochloric acid.*—Concentrated.

(b) *Standard iodine solution.*—0.04 *N*. 1 cc. of this solution is equivalent to 0.237 mg. of tin (Sn).

(c) *Sodium thiosulfate solution.*—Dissolve 6.0 grams of sodium thiosulfate in water, and dilute to 1 liter. Standardize with the 0.04 *N* iodine solution.

(d) *Antimony trichloride solution.*—Dissolve 0.5 gram of antimony trichloride in concentrated hydrochloric acid and add hydrochloric acid (1+1) to make 50 cc. volume.

(e) *Reduced iron.*

(f) *Carbon dioxide.*

(g) *Pieces of marble.*—About 1.5 grams each.

(h) *Standard tin solution.*—Dissolve 0.3 gram of tin foil in 65 cc. of concentrated hydrochloric acid and dilute to 1 liter at 20°C. with distilled water.

STANDARDIZATION OF IODINE SOLUTION

To 50 cc. of standard tin solution add 30 cc. of concentrated hydrochloric acid and 20 cc. of distilled water. Proceed according to methods (a) and (b).

[1] *This Journal*, 13, 423 (1930).

PREPARATION OF SOLUTION A-1

To 10 cc. of solution A in a 500 cc. volumetric flask, add 164 cc. of concentrated hydrochloric acid, mix, cool, and dilute to mark. Label bottle A-1 [50 cc. = 23.74 mg. of tin (Sn)].

Collaborative results

COLLABORATOR	SOLUTION	NO. OF DETERMINATIONS	METHOD	MILLIGRAMS OF TIN PER LITER MAX.	MIN.	AV.
A. Alter	A-1	4	a	490.4	485.1	488
		2	b	530.9	498.7	515
	B-1	4	a	85.6	82.5	84
		4	b	89.3	78.3	84
W. T. Mathis	A-1	4	a	482	480	480
		4	b	484	478	481
	B-1	4	a	99	97	98
		4	b	101	98	99
O. F. Mayer	A-1	–	a	unreported		455
		–	b	unreported		459
	B-1	–	a	unreported		75
		–	b	unreported		81
A. E. Mix	A-1	4	a	497	429	451
		3	b	544	483	507
	B-1	3	a	99	74	87
		2	b	95	46	66
W. D. Richardson	A-1	4	a	421.8	417.0	420
			b	unreported		
	B-1	4	a	152	30	83
			b	unreported		
G. C. Spencer	A-1	4	a	542.8	403.6	444
		4	b	581.4	492.2	528
	B-1	5	a	72.6	16.0	43
		4	b	98.0	74.4	87
A. H. Warth	A-1	4	a	499.2	495.2	497
		4	b	494.0	486.4	490
	B-1	4	a	97.3	76.8	88
		4	b	94.7	85.0	90

PREPARATION OF SOLUTION B-1

To 10 cc. of solution B in a 500 cc. volumetric flask, add 164 cc. of concentrated hydrochloric acid, mix, cool, and dilute to mark. Label bottle B-1 [50 cc. = 4.75 mg. of tin (Sn)].

DETERMINATION

Method a.—Measure 50 cc. of A-1 solution into a 200 cc. Erlenmeyer flask, add 2 drops of antimony trichloride solution and then 0.2 gram (approximately) of reduced iron powder, and heat until completely dissolved, keeping a small funnel in

the neck of the flask. Add a small piece of marble and cool the solution quickly to about 20°C. Keep funnel in the neck of the flask except while adding reagents.

Add an excess of 0.04 *N* iodine solution and titrate back immediately with the sodium thiosulfate solution. Calculate the results to milligrams per liter.

Method b.—Measure out 50 cc. of A-1 solution and proceed as in Method a to "Then add a small piece of marble." At this point conduct carbon dioxide into the flask through a two-holed rubber stopper while cooling. When cool, follow directions as given in Method a. Calculate results to milligrams per liter.

Repeat same procedure for solution B-1.

It is recommended[1] that the work on the determination of tin be continued.

REPORT ON COPPER

By REED WALKER (Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

In accordance with the recommendations made and approved at the last meeting, it was decided to have collaborators thoroughly test the proposed method[2] for determining copper in food. Twelve State and Federal chemists that ordinarily perform a similar type of work consented to cooperate on this project.

TABLE 1.
Strength of stock solutions used.

SUBSTANCE	ASH	STOCK SOLUTION	FORMULA OF COMPOUND ADDED
	per cent	*per cent*	
K_2O	32.0	0.96	KNO_3
Na_2O	2.0	0.06	$NaNO_3$
CaO	4.0	0.12	$CaCl_2$
MgO	9.0	0.27	$Mg(NO_3)_2 \cdot 6H_2O$
Fe_2O_3	2.0	0.06	$FeCl_3 \cdot 6H_2O$
H_3PO_4	38.0	1.14	85% H_3PO_4
H_2SO_4	2.0	0.06	95% H_2SO_4
Cl_2	2.0	0.06	Included as $CaCl_2$
SiO_2*	8.0	0.24	Na_2SiO_3
Cu	0.0633	0.00190	Pure copper foil dissolved in HNO_3
	99.0633	2.9719	

* Not all of the Na_2SiO_3 could be dissolved in the solution.

In order to provide a uniform sample for each analyst a stock solution was made. It contained the metallic salts commonly found in food products, including copper. A search of the literature showed that the average food material contains 3 per cent ash, which is composed of the

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 63 (1931).
[2] *This Journal*, 13, 426 (1930).

substances in the proportions indicated in Table 1. The percentage of the stock solution that each substance forms and the formula of compound added are also given in Table 1.

This solution simulates a food sample that has been ashed and the residue dissolved and made up to the original weight of sample. Although this method of providing a uniform sample does not test the method for ashing food materials, it does insure that all the analysts receive identical samples with which to test this method for determining copper.

A piece of pure copper foil and 500 cc. of the stock solution were furnished each collaborator. The method given on page 191 of the 1925 edition of *Methods of Analysis* was recommended for standardizing the sodium thiosulfate.

TABLE 2.

Collaborative results on copper.

COLLABORATOR	NO OF DETERMINATIONS	MAXIMUM DIFFERENCE IN DETERMINATIONS	MINIMUM DIFFERENCE IN DETERMINATIONS	AVERAGE DETERMINATION OF EACH COLLABORATOR
		mg. Cu per liter	*mg Cu per liter*	*mg. Cu per liter*
E. L. P. Treuthardt H. W. Haynes	2	None	None	18.72
J. W. Kellogg	1*	None	None	19.10
R. E. Remington	6	1.20	0.10	18.80
W. C. Geagley, M. M. Woodward	7	0.69	None	18.72
C. A. Greenleaf	3	0.60	0.20	18.33
E. M. Bailey, W. T. Mathis	8	0.80	None	20.10
Frederic Fenger	9	1.90	None	19.85
Geo. H. Marsh, E. K. Tucker	10	0.63	None	18.74
W. E. Kirby	4	4.60	0.20	17.50
I. Hochstadter	5	1.22	0.04	19.53
L. W. Mayer	5	1.37	0.24	17.48
P. P. Gray	4	2.20	0.30	16.30
	64	Av. 1.25 mg. Cu/L		

* Number of determinations made not given

The results submitted by the twelve collaborators as a whole are most satisfactory. They are uniform and near to the theoretical result, which is 19.00 mg. of copper per liter. The results are given in Table 2. The arithmetical average of the 64 determinations was found to be 18.82 mg. of copper per liter, this being only 0.18 mg. from the correct result, or 0.95 per cent error. The probable error for this group of determinations was ± 0.756 mg. of copper per liter.

This method appears to be reproducible, as is shown by columns three and four of Table 2. The average maximum difference between determina-

tions was only 1.25 mg. of copper per liter. By excluding the results of collaborator No. 9 this difference is reduced to 0.94 mg. of copper per liter. Each collaborator was able to obtain at least two results that corresponded within 0.30 mg. of copper per liter of each other.

The simplicity and ease with which a determination can be made by this method is best shown by some of the following voluntary comments of the collaborators. "The method appears to be simple, rapid, and gives reproducible results." "The proposed method is a great improvement over the present A.O.A.C. method in that it states the volume at which the sample should be kept before titration. * * * The method was also found workable and a time saver over the present method." "We found this method clear-cut and easy to handle. It can be run quickly and our analyst did not experience any difficulty in its execution." "The new method possesses the advantage that the filtration of the precipitated copper is very rapid." "On the whole, in spite of the difficulty experienced in obtaining a sharp end point when titrating with 0.01 *N* sodium thiosulfate, the method appears to be satisfactory and to give reproducible results."

The method used in standardizing the sodium thiosulfate solution is not considered entirely satisfactory for such dilute solutions as were used. The amount of copper specified for each titration is too large, and there is not sufficient limitation on the amount of water that can be used. Thus, the probable variation in concentration of the hydrogen- and iodide-ions may have caused a difference in the standardization factor with each collaborator. This fact is pointed out as a possible explanation for the great difference in results of one or two of the collaborators.

The associate referee wishes to express his sincere appreciation to the collaborators for their splendid cooperation.

It is recommended[1] that further collaborative work on food samples be done on the proposed method.

REPORT ON LEAD

By E. H. Berry (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

The recommendation for this year was that study be continued on methods for the determination of lead, especially as applicable to the determination of this element in spray residue. No report was made last year and so far as the writer knows no work has been done by the association on the determination of lead in spray residue, although it is believed that some independent work has been done by one or two members of the Food and Drug Administration.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 63 (1931).

Directions for the determination of this element in gelatine and in baking powders appear in the A.O.A.C. methods of analysis. Both of these methods include as one step the precipitation of the lead as sulfate. It is very generally recognized that the precipitation as lead sulfate is unsatisfactory in the estimation of small amounts of lead since lead sulfate is soluble to the extent of 40 mg. per liter, and while its solubility is greatly reduced by an excess of alcohol and sulfuric acid an appreciable amount is lost in the ordinary course of manipulation.

Lead is also determined electrolytically either as metallic lead or as lead dioxide. The electrolytic methods are generally recognized as fairly satisfactory, although in the presence of phosphates and sulfuric acid low results are obtained.

However, it has been found that lead may be precipitated and weighed as the chromate. The associate referee investigated the recovery of lead by this method, using a solution containing a known quantity of lead. The results obtained were entirely satisfactory. If it is not desired to weigh the lead chromate, it may be estimated volumetrically by dissolving the chromate in hydrochloric acid, adding an excess of potassium iodide, and titrating the liberated iodine with standard sodium thiosulfate solution. It is understood that there is also a colorimetric method, but the writer is not familiar with the details.

In the determination of lead in spray residue the most difficult part of the operation involves the preparation of the sample, that is, getting rid of the organic matter.

In reviewing the literature, an article, entitled "The Estimation of Minute Amounts of Lead in Biological Material," was found. It is by Lawrence J. Fairhall of the Harvard Medical School.[1] Fairhall prepared the material by ashing at a temperature well below a full red heat. The ash was taken up in tartaric acid to which a few drops of hydrochloric acid were added. This solution was neutralized with sodium hydroxide and then made faintly acid with hydrochloric acid. The lead was then precipitated as sulfide by passing hydrogen sulfide into the cold solution. The sulfide was filtered off and dissolved in nitric acid, and the nitric acid was neutralized with sodium hydroxide and then acidified with acetic acid. The lead was finally precipitated as the chromate.

While it did not seem possible to determine lead in spray residue by first ashing the sample, the method was considered worthy of a trial. Accordingly, some apple peelings were ground in a food chopper. Known amounts of lead were added to several portions of the ground peelings and well mixed. The material was then ashed in a muffle kept at a dull red heat, in fact, scarcely no redness could be detected in daylight. This temperature was very close to 500° C. The ash was then dissolved in nitric acid, the acid was neutralized with sodium hydroxide, then made just acid

[1] *J. Ind. Hyg.*, 4, 9(1922).

with hydrochloric acid, and the lead was precipitated as sulfide and finally weighed as chromate. The associate referee was much surprised to obtain very good results. Seven determinations gave the following results: 0.0053, 0.0061, 0.0053, 0.0051, 0.0052, 0.0056 and 0.0055 mg. of lead. In each case 0.0055 mg. of lead was added. It is not considered that a sufficient number of determinations have been made to prove that little or no loss of lead takes place by volatilization during the ashing process, but sufficient time was not available to make a larger number of determinations.

It is suggested, therefore, that this study be continued next year. There are many angles that may be taken up. It may be possible to use a part of the same solution for the determinations of lead as is used for the estimation of the arsenic in the spray residue. This is greatly to be desired and should be kept in mind in working out a method adaptable to the estimation of lead in spray residue.

It is recommended[1] that the study of lead in spray residues be continued.

REPORT ON FRUITS AND FRUIT PRODUCTS

By H. J. WICHMANN (U. S. Food and Drug Administration, San Francisco, Calif.), *Referee*

Subcommittee C recommended that work be done on the determination of plant bases, solids in sucrose and fruit or fruit acid mixtures, fruit acids, and the effect of a definite pH on the extraction of ash and pectin from fruit products as indicated by the ash, alcohol precipitate and pectic acid determinations.

F. A. Vorhes, Jr. of the Seattle Station made a beginning on the pH problem but later other fruit work demanded his time, therefore no report is made. The recommendation that this work be resumed is repeated.

The present tentative methods for the analysis of fruit products specify the extraction of ash and pectin from fruit or fruit products by boiling 300 grams with 800 cc. of water for 1 hour. The referee was curious to know whether there was any material difference between the amounts of ash, alcohol precipitate, or pectic acid extracted when sugar beyond that in the fruit itself was present. Fruits contain but a small quantity of sugar as compared with that found in jam. Salinger and Vorhes analyzed some representative fruits by the present methods and also after extraction with added sugar. The data obtained are given in Table 1.

The data given in the table show that the added sugar in some instances seems to have the power to extract slightly more ash material than the water alone. In the pectic acid values there seems to be little difference. The alcohol-precipitate results are slightly higher from the sweetened ex-

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 63 (1931).

TABLE 1.

Effect of sugar on extraction of ash and pectin from fruit.

FRUIT	EXTRACTION METHOD	pH AFTER EXTRACTION	pH AFTER DILUTION TO 2 LITERS	ASH	ALCOHOL PRECIPITATE	PECTIC ACID	ANALYST
				per cent	*per cent*	*per cent*	
Strawberries	75 grams of fruit 75 grams of sucrose made to 450 cc. with H_2O, boiled 1 hr., diluted to 1 liter	—	—	0.477	0.592	0.363	Vorhes
Strawberries	150 grams of fruit made to 450 cc. with H_2O, boiled 1 hr. diluted to 1 liter	—	—	0.453	0.537	0.343	Vorhes
Strawberries	150 grams of fruit 150 grams of sugar, 800 cc. of H_2O, boiled 1 hr., diluted to 2 liters	3.65	3.69	0.37	0.55	0.34	Salinger
Strawberries	300 grams of fruit 800 cc. of H_2O boiled 1 hr., diluted to 2 liters	3.59	3.66	0.37	0.50	0.33	Salinger
Raspberries	150 grams of fruit 150 grams of sugar, 800 cc. of H_2O boiled 1 hr., diluted to 2 liters	3.49	—	0.43	0.54	0.28	Salinger
Raspberries	150 grams of fruit 150 grams of H_2O 800 cc. of H_2O boiled 1 hr., diluted to 2 liters	3 56	—	0 43	0.53	0.27	Salinger
Crabapples	150 grams of fruit 150 grams of sugar, 800 cc. of H_2O boiled 1 hr., diluted to 2 liters	3.50	—	0.54	0.92	0.60	Salinger
Crabapples	150 grams of fruit 150 grams of H_2O 800 cc. of H_2O boiled 1 hr., diluted to 2 liters	3.56	—	0.50	0.91	0.60	Salinger

tracting medium. As the pectic acid values are much alike, the probability is that the sugar may serve to prevent some degradation of pectin during the evaporations rather than to extract more pectin. The differences are not great, but since the use of added sugar seems to have some slight advantage, the referee would urge that for the present, in the case of unsweetened fruits, 150 grams of fruit be extracted by a medium consisting of 150 grams of sugar dissolved in 800 cc. of water.

The Associate Referee on Solids in Fruit Products, V. B. Bonney, sent out some collaborative samples of jams and jellies with a request that soluble solids be determined by means of the refractometer and by drying after a hot water extraction of the soluble solids. The results obtained were so erratic that he concluded the samples were not uniform. Therefore no report was presented.

L. H. McRoberts, in the referee's laboratory, made some experiments on the determination of solids in known mixtures of sucrose and organic acids and on fruit juices with added known quantities of sucrose. The results obtained were preliminary in character, but the indication was that those obtained by the refractometer were closer to the truth than those obtained by the drying methods involving heat. Apparently two factors causing errors of opposite sign enter into the drying methods. When solutions containing organic acids are heated, some inversion takes place, and the soluble solids are increased by 5 per cent of the amount of sucrose inverted. This causes a positive error, as shown by McRoberts. He also noted that the acid and heat appeared to have a destructive action on the inverted sugar, particularly in concentrated solution. A partially inverted sucrose and organic acid solution had less reducing power after drying than before. The quantity of solids after drying was less than expected considering the original solids and the inversion of the sucrose. The results on solids by drying are believed by McRoberts and the referee to be a compensation of errors—one, a plus error due to inversion of sucrose by the acid, and the other a minus and generally smaller one, due to changes in the character of the invert sugar. Data are not presented here, because the referee intends to extend the experimental work and present a complete report next year.

The Associate Referee on Fruit Acids, B. J. Hartmann, and J. Hillig have published methods for the determination of citric and tartaric acids.[1] Collaborative results were very satisfactory. The referee therefore seconds Hartmann's recommendation that these methods be made tentative in place of the methods in the 1925 edition of *Methods of Analysis*. He also recommends that the work on fruit acids, particularly in reference to the malic acid methods, be continued. It is recommended that the methods for the determination of malic acid and the method for mixtures of malic and tartaric acids be dropped, because the former determines only

[1] *This Journal*, 13, 99, 103 (1931).

active malic acid, and the latter is very inaccurate, particularly if the malic acid is present in small quantities. It is hoped that Hartmann will soon be able to develop a method that will determine all forms of malic acid with an acceptable degree of accuracy.

The Associate Referee on the Determination of the Major Bases in Plant Ashes, Geo. T. Daughters, sent out collaborative samples containing potassium, manganese, calcium and magnesium in known amounts. The methods sent with the samples were developed from those proposed by the former associate referee, Mrs. Tilden. It was believed that the methods had been improved by the specification of the definite hydrogen-ion concentrations at which the precipitations were to be made. The whole scheme rests upon the progressive precipitation of the bases, except the alkalies, by the proper precipitant at the optimum *p*H for each base. The *p*H is gradually increased by means of buffer solutions. Iron and aluminum are precipitated as phosphates at the lowest *p*H. Then manganese, calcium and magnesium are successively separated at increasing ion concentrations. Special features of the methods are the avoidance of (1) the addition of ammonia or ammonium salts until nothing remains in solution but magnesium and the alkalies, and (2) the necessity of the removal of phosphates. Necessarily metals like copper, lead, tin or zinc are presumed to be absent, negligible or previously removed.

Some of the collaborative results reported by Daughters are very satisfactory, and some are disappointing. Experience is required before highly accurate results can be expected. The referee, however, believes that the methods are correct in theory and principle.

In reviewing the portion of Daughters' report relating to the theory of the method, the referee wishes to call attention to the reason given for the high results for manganese obtained by previous associate referees. Repeated ignitions and washings will remove the contaminating alkalies from the manganese dioxide but such a procedure is not practicable. Other methods for the determination of manganese, either colorimetric or volumetric, are available after it has been removed from solution and separated from the other plant bases.

The referee believes that Daughters' report on the single precipitation and determination of magnesium is a real contribution to analytical chemistry. The precipitation from a hot solution containing alkalies has been studied by many eminent chemists, but the results have always been slightly high. Hillebrand and Lundell[1] state unqualifiedly that accurate determinations for magnesium cannot be made without a double precipitation. The work of the associate referee seems to show that a single precipitation is practicable as well as accurate if care is taken first to obtain a *crystalline* precipitation of magnesium hydrogen phosphate. The photomicrographs prepared by G. L. Keenan are suggestive of the

[1] Applied Inorganic Analysis, 1929.

proper way to obtain accurate results. It can be easily understood from them why inaccurate results may be obtained by a poor crystallization. The secret of the precipitation lies in a *slow* precipitation at the critical pH.

The associate referee was not able to do much with the determination of iron and aluminum as the phosphates. Colorimetric methods are available for the determination of these elements in small quantities and likewise gravimetric or volumetric methods for large amounts, but there is need for a method that will determine these metals in foods in moderate amounts, that is, where the errors of a colorimetric method become excessive because of factor multiplications. Ash analysis of foods containing phosphorus requires the removal of iron and aluminum before other elements can be determined. A method for their determination as well as removal is desired. The referee believes the suggestions given by Daughters for washing the precipitated phosphates with a cold solution of volatile electrolyte to prevent hydrolysis, as well as a separation based on a controlled pH, should be followed by the next associate referee.

The referee also believes that the methods written by the associate referee for the determination of potassium, manganese, calcium and magnesium are correct in theory and principle and that sufficient collaborative work has been done by experienced analysts to warrant their adoption as tentative methods.

RECOMMENDATIONS[1]

It is recommended—

(1) That the study of the determination of soluble solids in fruit products, particularly the refractometric method, be continued.

(2) That a study be begun on the effects of a definite hydrogen-ion concentration of the extraction medium on the alcohol precipitate, pectic acid and ash of fruits and fruit products.

(3) That the present tentative methods for the determination of malic, citric and tartaric acids be dropped and that the methods for the determination of citric and tartaric acids published by Hartmann be substituted therefor.

(4) That the work on the determination of fruit acids, particularly malic acid, be continued.

(5) That the methods for the determination of potassium, manganese, calcium and magnesium reported by the associate referee be adopted as tentative.

(6) That the study of the determination of iron and aluminum and of chlorine in fruit products be continued.

No report on solids in solution of sucrose and organic acids was given by the associate referee. See report of Referee on Fruits and Fruit Products.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 63 (1931).

REPORT ON FRUIT ACIDS

By B. G. HARTMANN (U. S. Food and Drug Adm., Washington, D. C.), *Associate Referee*

This year's work on fruit acids was devoted to a collaborative study of methods for the determination of citric and tartaric acids in fruits and fruit products.

The methods investigated were those for tartaric acid as acid potassium tartrate[1] and for citric acid as pentabromacetone.[2]

Two samples of apple jelly prepared from Winesaps, to which known quantities of citric and tartaric acids had been added, were sent out An examination by the methods under investigation showed this jelly to contain neither citric nor tartaric acid.

The collaborators were instructed to use the entire contents of the sample container (300 grams of jelly) for the preparation of the sample solution and to report results in milligrams per 200 cc. of the sample solution. They were also advised to use 25 cc. instead of 50 cc. of the potassium permanganate solution in the oxidation of citric acid.

The following analysts participated in the study: L. A Salinger and W. C. Taber, San Francisco; R. L. Horst, New Orleans; F. A. Vorhes, Seattle; L. Jones, Kansas City; C. H. Hickey and P. L. Leavitt, Boston; and J. I. Palmore, P. A. Clifford, F. Hillig and B. G. Hartmann of the Food Control Laboratory, Washington, D. C.

The results reported by the collaborators are given in the table.

COMMENTS BY THE COLLABORATORS

Generally speaking, no unfavorable comments were received from any one of the collaborators on either of the two methods.

W. C. Taber, using the vacuum desiccator for drying the pentabromacetone, reported 88.3 mg. of citric acid in Sample No. 1 and 23.3 mg. in Sample No. 2.

Llewelyn Jones used the vacuum desiccator and experienced trouble with the aspiration method.

L. A. Salinger commented as follows: "Preliminary tests were made on crucibles to determine the time necessary for drying to constant weight.* * * The thickness of the asbestos was determined by adding asbestos until, when held to the light with the fingers behind it and looking through it, no shadow was apparent."

COMMENTS BY THE ASSOCIATE REFEREE

With the exception of the low results reported by Collaborators 4 and 5 for tartaric acid on sample No. 1, the results tabulated are very gratifying.

[1] *This Journal*, 13, 104 (1930).
[2] *Ibid.*, 99.

Collaborative results on tartaric acid and citric acid in apple jelly.

ANALYST	SAMPLE NO. 1			
	TARTARIC ACID		CITRIC ACID	
	SAMPLE SOLUTION	ORIGINAL	SAMPLE SOLUTION	ORIGINAL
	mg.	*per cent*	*mg.*	*per cent*
1	24.0	0.080	97.8†	0.326
	27.1	0.090	98.4†	0.328
	Av. 25.6	Av. 0.085	Av. 98.1	Av. 0.327
2	25.0	0.083	97.3	0.324
	18.1	0.060	96.1	0.320
	Av. 21.6	Av. 0.072	Av. 96.7	Av. 0.322
3	26.0	0.087	90.7	0.302
	27.1	0.090	92.0	0.307
	Av. 26.6	Av. 0.089	Av. 91.4	Av. 0.305
4	9.4*	0.031*	96.3	0.321
	9.4*	0.031*	96.3	0.321
	Av. 9.4	Av. 0.031	Av. 96.3	Av. 0.321
5	19.4*	0.065*		
	11.5*	0.038*	95.4	0.318
	Av. 15.5	Av. 0.052		
6	25.0	0.083	93.1	0.310
	25.0	0.083	91.2	0.304
	22.5	0.075		
	Av. 24.2	Av. 0.080	Av. 92.2	Av. 0.307
7	21.7	0.072	93.5	0.312
			92.9	0.310
	Av.		Av. 93.2	Av. 0.311
8	23.0	0.077	97.8	0.326
	22.0	0.073	95.6	0.319
	Av. 22.5	Av. 0.075	Av. 96.7	Av. 0.322
9	28.1	0.094	93.4	0.311
	26.0	0.087	94.8	0.316
	Av. 27.1	Av. 0.090	Av. 94.1	Av. 0.314
10	25.0	0.083	92.9	0.310
			96.6	0.322
		Av.	Av. 94.8	Av. 0.316
11 (Referee)	24.0	0.080		
	22.0	0.073	97.6	0.325
	Av. 23.0	Av. 0.077		
General average	24 2	0.081	95.0	0.317
Sample contained	26.7	0.089	98.9	0.330

* Not included in the general average.
† Pentabromacetone dried in vacuum desiccator.

ANALYST	SAMPLE NO. 2			
	TARTARIC ACID		CITRIC ACID	
	SAMPLE SOLUTION	ORIGINAL	SAMPLE SOLUTION	ORIGINAL
	mg.	*per cent*	*mg.*	*per cent*
1	95.8	0.319	26.4†	0.088
	95.8	0.319	24.8†	0.083
			24.9†	0.083
	Av. 95.8	Av. 0.319	Av. 25.4	Av. 0.085
2	97.9	0.326	22.6	0.075
	95.6	0.319	21.5	0.072
	Av. 96.8	Av. 0.323	Av 22.1	Av. 0.074
3	94.8	0.316	23.9	0.080
	94.8	0.316	24.2	0.081
	Av. 94.8	Av. 0.316	Av. 24.1	Av. 0.081
4	97.3	0.324	24.3	0.081
	97.3	0.324	24.4	0.081
	Av. 97.3	Av. 0.324	Av. 24.4	Av. 0.081
5	93.3	0.311		
	95.6	0.319	26.0	0.087
	Av. 94.5	Av. 0.315		
6				
7	101.0	0.337	22.8	0.076
	100.0	0.333	23.3	0.078
	Av. 100.5	Av. 0.335	Av. 23.1	Av. 0.077
8	97.9	0.326	26.1	0.087
	100.0	0.333	26.2	0.087
	Av. 99.0	Av. 0.330	Av. 26.2	Av. 0.087
9	92.7	0.309	22.8	0.076
	96.9	0.323	23.7	0.079
	Av. 94.8	Av. 0.316	Av. 23.3	Av. 0.078
10	95.8	0.319	21.6	0.072
	96.9	0.323	22.2	0.074
	Av. 96.4	Av. 0.321	Av. 21.9	Av. 0.073
11 (Referee)	100.1	0.334	26.0	0.087
	100.0	0.333	26.1	0.087
	Av. 100.1	Av. 0.334	Av. 26.1	Av. 0.087
General average	97.0	0.323	24.2	0.081
Sample contained	100.7	0.338	25.3	0.084

The determination of small quantities of tartaric acid as acid potassium tartrate requires careful attention, particularly with regard to the precipitation and the subsequent washing of the tartrate. The mixture, after chilling, should be stirred vigorously for the 2 minutes prescribed and the wash solution should be *ice cold.*

The results obtained by the collaborators on citric acid are also satisfactory. The drying of the pentabromacetone by the aspiration method offers no difficulty if the asbestos in the crucible is thin and tightly tamped. It is quite possible that the trouble experienced by Collaborator Jones was due to heavy pads. A good way to determine the proper thickness of the pad is that suggested by L A. Salinger. It is of the greatest importance to the proper drying of the pentabromacetone that the surface moisture be removed before the cup is placed in the apparatus. This is accomplished by allowing the cup to remain under suction about 1 minute before proceeding with the aspiration. Occasionally the pentabromacetone is in such fine condition that the air does not pass through it freely, in which case the cup should be placed in a desiccator for a short time. No definite instructions can be given regarding the rate of aspiration, except to crack the suction so that the air passes through *slowly* and uniformly. It should be remembered that pentabromacetone is volatile at low temperature, and therefore the temperature should be kept as low as possible during drying. If the apparatus is properly adjusted, drying should be complete in 20 minutes, and an additional aspiration of 5 minutes should not show more than a loss of a few tenths of a milligram.

MALIC ACID

The work conducted on methods for the determination of malic acid has not progressed far enough to warrant definite recommendations on directions for a procedure. The studies made show that a number of difficulties arise with the present tentative procedure and that these are not due to an inaccuracy in the principle of measuring the increased rotation of malic acid through the use of uranium salts. The procedure is not applicable to the determination of synthetic malic acid, which is inactive. It is now an article of commerce and used in the food industries For the purpose of revision of *Methods of Analysis* it is suggested, therefore, that the method for the determination of malic acid be dropped.

RECOMMENDATIONS[1]

It is recommended—

(1) That the method for the determination of tartaric acid, sec. **18,** p. 213, chap. XIV, be dropped.

(2) That the method for the determination of citric acid, sec. **24,** p. 215, be dropped.

[1] For report of Subcommittee C and action of the association, see *This Journal,* 14, 64 (1931).

(3) That the method for the determination of malic acid, secs. 19–23, pp. 213–215, be dropped.

(4) That the method for the determination of tartaric acid as acid potassium tartrate, published previously,[1] be adopted as a tentative method.

(5) That the method for the determination of citric acid, published previously,[2] be adopted as a tentative method.

(6) That further study be made of the methods for the determination of fruit acids.

REPORT ON ASH IN FRUIT PRODUCTS

By GEORGE T. DAUGHTERS (U. S. Food and Drug Administration, San Francisco, Calif.), *Associate Referee*

The work this year on the determination of the bases of fruit ashes is a continuation of that reported by Doris H Tilden[3] in 1928. Particular attention was given to the pH at which precipitations are made. The methods Tilden recommended depend upon the precipitation of iron and aluminum as the phosphates in acetic acid solution, precipitation of manganese and calcium in a more dilute acetic acid solution without the removal of phosphates or the addition of ammonium compounds, until the final precipitation of the magnesium. Very good results were reported on the determination of all the bases except those from the gravimetric manganese method. Since 1928 analysts at the San Francisco station have continued to work on the problem of determining bases of plant ashes. Quick Landis, formerly of this station, suggested a single precipitation method for calcium, in which oxalic acid is used as the precipitant. The following data are representative of the results that may be expected by the use of the methods reported by Tilden.

The method for determining the quantities of potash likely to be found in plant ashes, on the basis of the above data, seems to be all that could be desired and was therefore included without change in the methods sent to collaborators. Because the gravimetric results for manganese, like those obtained by Tilden, were high, the associate referee decided to omit this method, and to send out with the collaborative samples instructions for the colorimetric method only. Later he obtained evidence which explained the high gravimetric results. The data on the double precipitation of calcium in Table 1 appear to be very good, although there is one low result, probably due to mechanical losses. The original single precipitation method appeared to require modification, since the results were invariably high. Most of the magnesium results were a trifle high. Because Tilden

[1] *This Journal*, 13, 104 (1930).
[2] *Ibid.*, 99.
[3] *Ibid.*, 12, 362 (1930).

does not mention the hydrogen-ion concentration at which her precipitations were made, the associate referee modified her methods to incorporate a statement of the optimum *p*H at which the precipitations should be made, and a means of obtaining the same.

TABLE 1.

Collaborative results for ash obtained at San Francisco Station.
(Solutions prepared by D. H. Tilden)

ANALYST	Ash Constituents (mg. per 100 cc.)							
	K_2O			Mn_3O_4		CaO		MgO
	Sol. A	Sol. B	Sol. C	GRAV.	COLOR.	SINGLE PPT.	DOUBLE PPT.	
G. T. Daughters	90.92	91.19	109.26 110.42	9.4 9.1	8.7 8.3		103.45 104.6	101.33 102.33
T. O. Kellems	93.7	—	—	8.5 9.1	8.1 8.3	105.8	96.8	96.08 101.67
Q. Landis				8.3 9.7	9.1 9.7	105.6 103.8	104.2	102.3 102.6
L. H. McRoberts	92.6	94.2	111.1 110.7	10.6 11.6	9.1 9.0	108.4	104.0	103.4 102.2
L. A. Salinger	90.3	90.0		11.1 12.0	7.1 7.7	107.6 106.8	103.4 104.8	102.3 98.4
W. C. Taber	92.0	—	110.3 109.2	10.5 10.2	7.7 8.1	105.0	101.1	103.5 104.3
R. Jinkins	88.8	90.2						
Theory	91.6	91.6	110.79	8.9	8.9	104.04	104.04	100.0
Average	91.39	91.39	110.1	10.0	8.5	105.65	102.86	101.56
Maximum	93.7	94.2	111.1	12.0	9.7	108.4	104.8	104.3
Minimum	88.8	90.0	109.2	8.3	7.1	103.8	96.8	96.08

A series of collaborative solutions was carefully prepared from recrystallized potassium chloride, potassium permanganate, reprecipitated calcium oxalate ignited to the oxide, and magnesium ribbon,—all dissolved in hydrochloric acid. The solutions also contained small quantities of iron and aluminum and a moderate quantity of sodium hydrogen phosphate.

The solutions were sent to collaborators with the following instructions:

PROPOSED METHODS FOR THE DETERMINATION OF POTASSIUM, MANGANESE, CALCIUM, AND MAGNESIUM IN PLANT ASHES

POTASSIUM[1]

REAGENTS

(a) *Ammonium chloride solution.*—Dissolve 100 grams of ammonium chloride in 500 cc. of water, add 5–10 grams of pulverized potassium platinic chloride, and shake at intervals for 6–8 hours. Allow mixture to settle overnight and filter. (The residue may be used for the preparation of a fresh supply.)

(b) *Platinum solution.*—For materials containing less than 15 per cent of potash, a platinic chloride solution containing 0.2 gram of metallic platinum (0.42 gram of H_2PtCl_6) in each 10 cc. is recommended.

(c) *90 per cent alcohol.*—Sp. gr. 0.8339 at 15.6°/15.6°C.

PREPARATION OF SOLUTION

(A) Dissolve the ash in hydrochloric acid. If it is desired to take an aliquot, filter into a volumetric flask, wash filter thoroughly, and make up to volume. Pipet an aliquot into a beaker, adjust to a volume of 50–75 cc., heat to boiling, and add a slight excess of strong ammonium hydroxide and then sufficient saturated ammonium oxalate solution to precipitate all the lime and aluminum present. Filter into a large platinum dish and wash filter thoroughly.

DETERMINATION

Evaporate solution from (A) nearly to dryness, add 1 cc. of sulfuric acid (1+1), evaporate to dryness, and ignite to whiteness. Maintain a full red heat until the residue is perfectly white. Dissolve the residue in hot water, using at least 20 cc. for each decigram of potassium oxide present; add a few drops of strong hydrochloric acid and then an excess of reagent (b). Evaporate on a water bath to a thick paste, avoiding exposure to ammonia. Treat the residue with 90 per cent alcohol. Filter,[2] wash the precipitate thoroughly with 90 per cent alcohol, both by decantation and on the filter, continuing the washings after the filtrate is colorless, using about 200 cc. of wash solution. Then wash 5 or 6 times with 10 cc. portions of the ammonium chloride solution to remove impurities from the precipitate. Wash again with four or five 10 cc. portions of 90 per cent alcohol and dry the precipitate for 30 minutes at 100°C. Weigh, wash again with several 10 cc. portions of 90 per cent alcohol, dry, and reweigh until a constant weight of platinic chloride is obtained. Calculate to potassium oxide. The precipitate should be completely soluble in water.

MANGANESE, CALCIUM AND MAGNESIUM

REAGENTS

(a) *Sodium hydroxide solution.*—10%, freshly prepared.

(b) *Sodium oxalate solution.*—Saturated.

(c) *Saturated oxalic acid.*[2]

(d) *Sodium acetate solution.*—20 per cent.

(e) *Sodium ammonium phosphate solution.*—$NaHNH_4PO_4$, 10 per cent.

(f) *Ammonium hydroxide.*—100 cc. of concentrated NH_4OH diluted to 1 liter.

(g) *Hydrochloric acid.*—5 cc. of concentrated HCl diluted to 30 cc.

(h) *Sodium dihydrogen phosphate.*—10 per cent.

(i) *Ammonium oxalate solution.*—Saturated.

[1] Method proposed by D. H. Tilden, *This Journal*, 12, 366 (1929).

[2] See recommendations at end of this report.

PREPARATION OF SOLUTION

(B) Dissolve the ash in hydrochloric acid, evaporate to dryness, and bake at 110° for 1 hour to dehydrate the silica. Dissolve the residue in dilute HCl and filter into a volumetric flask. Wash the filter thoroughly, and make up to volume.

DETERMINATION

Manganese

To an aliquot of solution B add sufficient bromine water to oxidize any ferrous iron to the ferric state. Boil off the excess bromine. Dilute to 150 cc. and heat to boiling. Add a sufficient quantity of reagent (h) to combine with all the iron and aluminum present. Add plenty of bromcresol green indicator and while gently boiling add sodium hydroxide (a) dropwise to the first permanent turbidity or an initial color change in the event no iron or aluminum compounds are present. Continue neutralization by slowly adding sodium acetate (d) to give a yellow-green color. Iron and aluminum phosphates are completely precipitated at a *p*H of 4 (at which point bromcresol green indicator is yellow-green—see color chart in Clark's "The determination of Hydrogen Ions"). Boil gently for 1–2 minutes if any precipitate of aluminum or iron phosphate forms. Allow to settle, filter, and wash carefully. Discard the precipitate.[1] To the filtrate add 10 cc. of sodium acetate (d) and adjust the *p*H to 4.2–4.4 (indicated by a yellow-green color with bromcresol green indicator) by adding dilute hydrochloric acid (g) dropwise. Add sufficient bromine water to color the solution distinctly orange, cover with a watch-glass and boil gently for about 3 minutes. Great care must be taken to avoid bumping. Allow to settle, filter, and wash beaker and filter thoroughly. The filtrate is reserved for calcium and magnesium determinations. The manganese is determined colorimetrically by the periodate method. Dissolve the hydrated oxide precipitate from the filter into the original beaker with as little solution of water saturated with sulfur dioxide as possible. Wash the filter paper thoroughly with hot water. Boil to remove all odor of sulfur dioxide, and add 10 cc. of concentrated sulfuric acid and 10–20 cc. of concentrated nitric acid. Yoe[2] recommends that the amount of manganese in the final dilution for colorimetric comparison be no more than 1.0 mg. per 50 cc. Very accurate results may be expected. Report as percentage of Mn_3O_4 by multiplying $KMnO_4$ by factor 0.4827.

CALCIUM

(1) *Double precipitation method.*[3]

Evaporate the filtrate from the manganese determination to 100–150 cc. Boil off any bromine remaining and adjust the *p*H to 4.4–4.6 (green to green-blue with bromcresol green indicator) by adding 20 per cent of sodium acetate (d). A *p*H of 4.4–4.6 is the most favorable for precipitation of calcium oxalate. Add sufficient saturated sodium oxalate solution (b) dropwise to precipitate all the calcium from the boiling solution. Continue to boil until the oxalate begins to settle, or digest for 15 minutes on the steam bath. Allow to settle until clear, filter, and wash the precipitate thoroughly with hot water. Reserve the filtrate and washings for the magnesium determination. Carefully wash the precipitate back into the original beaker, heat, and dissolve the oxalate by adding as little concentrated hydrochloric acid as possible. Reprecipitate the calcium by adding dilute ammonium hydroxide dropwise until the *p*H is again 4.4–4.6 (green to green-blue with brom cresol green indicator). Add a slight excess of saturated ammonium oxalate solution (i) while still hot. Digest

[1] Methods for the determination of iron and aluminum as phosphates are in preparation.
[2] Photometric Chemical Analysis, p. 274 (1928).
[3] The double precipitation method is essentially that worked out by D. H. Tilden, *This Journal*, 12, 367 (1929).

on a steam bath for 1 hour and set aside until the supernatant liquid is clear, preferably overnight. Filter and wash with hot water. Determine the calcium either gravimetrically or volumetrically by the usual methods. (For small quantities of calcium the gravimetric method is preferred.) Report as CaO.

If magnesium is not to be determined, precipitate the calcium once from the boiling solution freed from iron, aluminum and manganese with saturated ammonium oxalate solution (i), digest, and determine as described.

(2) *Single precipitation method.*[1]

Evaporate the filtrate and washings from the manganese determination to 200–250 cc. Add 8–10 drops of bromcresol green and sufficient sodium acetate (d) to change the *p*H to 4.8–5.0 (blue). Cover with a watch-glass and heat to boiling. Precipitate the calcium slowly by adding dropwise saturated oxalic acid solution (c) sufficient to change the *p*H back to 4.4–4.6 (the optimum for calcium oxalate precipitation) as indicated by the appearance of a distinct green shade. The change in color will indicate an excess of oxalic acid—more would develop yellow tints, showing an undesirable displacement of the *p*H. Boil 1–2 minutes and allow to settle until clear. Filter and wash thoroughly with hot water. Determine either gravimetrically or volumetrically as in the double precipitation method.

MAGNESIUM

Add 2–3 drops of concentrated hydrochloric acid to the filtrate and washings from the calcium determination and evaporate to 75–100 cc. Heat to gentle boiling and neutralize with dilute (10%) ammonium hydroxide until the first permanent precipitate forms. Boil gently for ½ minute, then add dropwise an excess of 10 per cent sodium ammonium phosphate (e), and make slightly ammoniacal with dilute ammonium hydroxide (f) added slowly and with constant stirring. Boil for 2–3 minutes. This treatment gives an amorphous precipitate of magnesium hydrogen phosphate ($MgHPO_4$). Allow to cool slightly, then add ⅓ the volume of concentrated ammonium hydroxide slowly, and with constant stirring. Let stand until the precipitate is crystalline, preferably overnight. Filter and wash carefully with dilute ammonium hydroxide (f) until all chlorides have been removed. Dry and ignite slowly until all the carbon is consumed; cover with a lid and ignite intensely. Weigh the white magnesium pyrophosphate ($Mg_2P_2O_7$) and report as MgO. ($Mg_2P_2O_7 \times 0.3621 = MgO$.) Ignition of dark colored residues with a drop of 20 per cent ammonium nitrate will often improve the color. If the nitrate is added, use care to avoid spattering.

The results obtained are given in Table 2.

Some of the comments of the collaborators follow:

A. G. Buell.—It has been my experience in the determination of potassium that * * * aluminum oxide is weighed with the potassium chloroplatinate, and it is therefore essential that the precipitate be entirely water-soluble.

J. H. Cannon.—The magnesium precipitate remained bulky overnight and became crystalline only after more HCl was added.

N. L. Knight.— * * * in the double precipitation of calcium a saturated solution of ammonium oxalate was used in place of a saturated sodium oxalate. * * * During the course of every magnesium determination trouble was experienced at the point "boil for 2–3 minutes" due to the occurrence of violent bumping, * * * might not digestion on a steam bath with frequent stirring for a longer period be substituted.

C. H. Badger.— * * * I have interpreted that the K_2PtCl_6 is weighed on the filter paper. I have never been able to obtain constant weights when it is necessary to weigh filter papers with precipitates.

[1] Based on a suggestion of Quick Landis, formerly of the San Francisco Station.

TABLE 2.

Collaborative results for the determination of major bases in plant ashes.
(Expressed as mg. per 100 cc.)

ANALYST	K_2O		Mn_3O_4	CaO		MgO	
	A	B		SINGLE PPT. METHOD	DOUBLE PPT. METHOD	AFTER SINGLE PPT. OF CaO	AFTER DOUBLE PPT. OF CaO
			Solution 1				
Theory	41.67	83.34	8.05	93.3	93.3	100	100
J. H. Cannon			7.0 7.5	93.0	91.0	84.0	
D. Dahle	40.49	80.60	6.6 6.3	104.0	92.0	103.7	102.3
G. T. Daughters	41.76	82.75	8.0 8.2	93.9	93.2	99.4	101.4
N. L. Knight	45.4	30.8	7.7	101.5	120.0	103.2	105.7
C. H. Badger	48.7	83.6	6.9 7.1	93.8	90.0	101.6	105.7
			Solution 2				
Theory	41.67	83.34	8.05	113.4	113.4	100.0	100.0
A. G. Buell	41.0	83.0	8.4 8.7	113.4	113.0	103.1	103.4
G. T. Daughters				114.0	110.0	99.58	99.0
N. L. Knight	58.1	37.2	7.64 7.75	165.0	123.0	101.0	95.6
W. C. Taber			8.3 7.2	113.7	111.2		106.7
L. G. Petree	42.17	83.95	8.25 8.4	114.1	109.6	100.74	101.8
			Solution 3				
Theory	41.67	83.34	8.05	110.04	110.04	100.0	100.0
J. H. Cannon	46.0	85.0	6.7 4.6		104.9		95.0

This work seems to have been a test of analysts as well as of methods. The analysts that have had considerable experience with these methods seem to have done better work than those without any experience. The indicator seems to have offered some difficulty. Instead of comparing

with the Clark color chart, it might be better for the analyst to prepare some solutions of known *p*H, with the indicator present. A direct comparison between solutions might possibly be easier.

The associate referee also experienced bumping in both the manganese and magnesium determinations. However, very careful maneuvering in the former method and the use of the method for magnesium given later in this report solve these difficulties to a large extent. It is understood that when using these methods no ammonia (or ammonium salts) will be introduced previous to the precipitation of magnesium. However, as stated previously, experience is indispensable in adapting these methods to fruit ashes if accurate results are to be expected.

WORK OF ASSOCIATE REFEREE

After the collaborative samples had been sent out, the associate referee worked on the gravimetric determination of manganese, the determination of magnesium, which yielded high results generally, and the gravimetric determination of iron and aluminum as the phosphates.

Tilden attributed the high gravimetric results for manganese to the contamination of Mn_3O_4 with Mn_2O_3. The associate referee concluded, however, that these high results were due largely, if not wholly, to the contamination of Mn_3O_4 with alkalis. When the ignited oxide was dissolved in dilute sulfuric acid and a reducing agent, such as sulfur dioxide or hydrazine sulfate, was added to convert the oxide to the sulfate, and the resulting solution was evaporated without spattering and ignited at 500°C., the sulfate results tallied very closely with the oxide results.

By washing the ignited Mn_3O_4 with water, and reigniting, there was a loss in weight, but no manganese was detected in the washings tested by the delicate periodate method. Several washings with hot water, alternated with ignition, gradually reduce the Mn_3O_4 to the theoretical, but this process proved to be very tedious. For example, 12.07 mg of Mn_3O_4, when precipitated and ignited, weighed 13.5 mg., and this quantity was reduced to 12.9 mg., then 12.4 mg., and finally 12.1 mg. by successive washings and ignitions. It was hoped that dilute acetic acid could be used to accomplish this purpose in one washing, but it was found that when acetic acid was used part of the manganese was dissolved.

As both the gravimetric sulfate and oxide methods yielded similar high results, and washing of the oxide with water reduced the weight but showed no removal of manganese, it was concluded that the high results were probably due to contamination with sodium salts derived from the sodium acetate buffer present.

Some of the gravimetric data obtained from solutions containing known quantities of manganese are given in Table 3.

That the sulfate results are practically equivalent to the oxide results in each case appears to be conclusive evidence that Tilden's high results

TABLE 3.

Results of manganese determinations by the gravimetric method.

THEORY Mn_3O_4	DETERMINED AS Mn_3O_4		REDUCING AGENT	DETERMINED AS $MnSO_4$	
mg.	*mg.*	*per cent*		*mg.*	*per cent*
10.88	12.5	114	Hydrazine sulfate	12.43	111
10.88	12.9	118	Hydrazine sulfate	12.95	119
10.88	12.5	114	Sulfur dioxide	12.97	119
10.88	13.0	119	Sulfur dioxide	12.97	119

were due to some contamination of Mn_3O_4 other than higher oxides of manganese. It would also seem that the only hope for the gravimetric determination of manganese as the oxide lies in reprecipitating the redissolved MnO_2 with a strong oxidizing agent in the presence of buffers which will have no non-volatile residues. A suitable organic oxidizing agent might be successful. Hydrogen peroxide cannot be used because it is decomposed as soon as a small amount of MnO_2 is formed, due probably to the catalytic effect of MnO_2. Since the gravimetric method is unsuccessful, it appears to the associate referee that the method used for determining manganese in fruit ashes after separation from other bases will depend on the quantity of manganese present. The colorimetric method will probably meet the requirements in most cases, but it is limited to small quantities, hence for samples containing larger quantities the bismuthate method should be used.

MAGNESIUM

Most of the magnesium results were slightly high. A single precipitation of magnesium hydrogen phosphate from a boiling solution and subsequent conversion into the magnesium ammonium phosphate has often been recommended. However Hillebrand and Lundell[1] recommend double precipitation in all cases if accurate results are to be expected. Also, the experience of the analysts at the San Francisco Station and of other collaborators indicates that when fair amounts of magnesium are present in the solution with a high concentration of alkali salts, some contamination does occur. The associate referee was reluctant to recommend the double precipitation method, especially since the single precipitation method for calcium, when carefully conducted, gave such excellent results. Magnesium hydrogen phosphate has been described as amorphous, and as crystalline. This conflict of opinion directed suspicion toward precipitation as usually conducted, and the resultant non-crystalline, more or less gelatinous precipitate, described as magnesium hydrogen phosphate. A microscopic examination of this precipitate showed it to be of uncertain structure, similar to a suspension of agar agar. While it is true that this amorphous precipitate is converted to crystalline magnesium ammonium phosphate upon standing with strong ammonia, but small quantities of

[1] Applied Inorganic Analysis, 509.

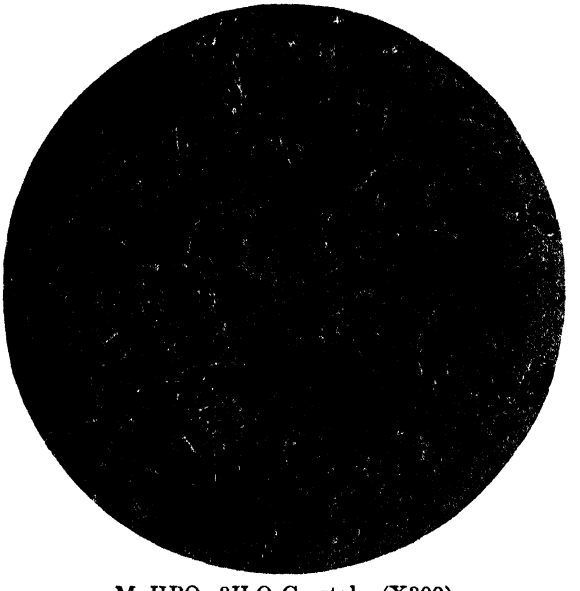
foreign material entangled in the gelatinous precipitate might easily be held in the crystals of magnesium ammonium phosphate formed therefrom. It therefore seemed wise to determine under what conditions a crystalline magnesium hydrogen phosphate would be formed. In a description of the precipitation of phosphoric acid, Treadwell and Hall direct that the magnesium hydrogen phosphate be precipitated from a solution slightly acid and containing an excess of magnesia mixture by adding 2.5 per cent ammonia very slowly (4 drops to the minute). This procedure produces a crystalline precipitate when applied to the precipitation of magnesium, but the first precipitate must be crystalline, not gelatinous. When a gelatinous precipitate forms at first, the associate referee found that it was necessary to dissolve the precipitate by adding a few drops of dilute hydrochloric acid and to reprecipitate more slowly. Precipitation of crystalline magnesium hydrogen phosphate commences at a *p*H of 6.7–6.8—just the acid side of neutrality. A little experience enables the analyst to know when to expect the first precipitate, which forms very slowly. The solution is gently and continuously boiled. Stirring assists in crystal formation, but care must be taken to avoid scratching the beaker. A rubber cap on the stirring rod, such as a new, clean, magnesium-free policeman, is very effective. Once crystallization is well started the ammonia may be added more rapidly. The first crystallization is the critical point. If the magnesium is precipitated as this crystalline hydrogen phosphate, it is evidently very pure, and is converted by the ammonia into the ammonium phosphate without incorporated impurities.

On examining a number of preparations of crystalline magnesium hydrogen phosphate under the microscope it was found that the crystal habit varied. Needles and plate-like crystals have been observed. The typical crystals seemed to be rhombohedral or diamond shaped. George L. Keenan of the Microanalytical Laboratory of the U. S. Food and Drug Administration called attention to a statement by Mellor[1] to the effect that magnesium hydrogen phosphates did exist as $MgHPO_4 \cdot nH_2O$, where n is 1, 3, $4\frac{1}{2}$ or 7. Variation in the number of molecules of water of crystallization may perhaps be the reason for the various shapes of the crystals observed. Keenan prepared two photomicrographs from specimens of magnesium hydrogen phosphate and magnesium ammonium phosphate crystals supplied him, which he concluded to be most typical. These photomicrographs are shown on the accompanying plate. He also reported that he was able to confirm the identity of these salts by the optical immersion method as being $MgHPO_4 \cdot 3H_2O$ (identical with the mineral Newberyite) and $MgNH_4PO_4 \cdot 6H_2O$ (identical with Struvite).

A few of the data obtained by the associate referee with this method of precipitation are given in Table 4.

[1] A Comprehensive Treatise on Inorganic Chemistry, Vol IV, p 390

These results are within one per cent of the theoretical, and are all that could be desired. The associate referee therefore recommends that the method for the determination of magnesium be modified to include the crystalline precipitation method and it should be written as follows:



$MgHPO_4 \cdot 3H_2O$ Crystals. (X300)



$MgNH_4PO_4 \cdot 6H_2O$ Crystals. (X300)

MAGNESIUM

Add 2–3 drops of concentrated hydrochloric acid to the filtrate and washings from the calcium determination and evaporate to 75–100 cc. If the quantity of phosphates naturally in the sample, or added for the purpose of precipitating iron and aluminum, is insufficient to precipitate all the magnesium expected, more must be added but a large excess should be avoided. For this purpose heat the solution to gentle boiling and neutralize with dilute (10%) ammonium hydroxide until a permanent precipitate forms. Add sufficient sodium hydrogen phosphate (e) solution to precipitate all magnesium present. Dissolve the precipitate by slowly adding 10 per cent hydrochloric acid dropwise. Use as little hydrochloric acid as possible to obtain complete solution. The next step requires considerable care and patience to give accurate results. Magnesium hydrogen phosphate begins to precipitate at a *p*H of 6.7–6.8. This is the critical point. Add dilute ammonium hydroxide (f) at the rate of 4 drops a minute while maintaining a gentle boil until a crystalline precipitate commences to form. [The first precipitate must be crystalline, not gelatinous. If the first precipitate is gelatinous it is necessary to redissolve it with a little hydrochloric acid and start the precipitation again more slowly. Stirring assists crystallization but the sides of the beaker should not be scratched. After the crystals have formed in considerable numbers the precipitation may be speeded up. This treatment gives crystalline magnesium hydrogen phosphate ($MgHPO_4$).] Continue the addition of the dilute ammonia until the solution is slightly ammoniacal. Allow to cool slightly, then add ⅓ the volume of concentrated ammonium hydroxide slowly and with constant stirring. Let stand until the precipitate has been converted into magnesium ammonium phosphate, preferably overnight. Filter and wash carefully with dilute ammonium hydroxide (f), until all chlorides have been removed. Dry and ignite the precipitate slowly until all the carbon is consumed; cover with a lid and ignite intensely. Weigh the white magnesium pyrophosphate ($Mg_2P_2O_7$) and report as MgO ($Mg_2P_2O_7 \times 0.3621 = MgO$). (Ignition of dark colored residue with a drop of 20 per cent ammonium nitrate will often improve the color. If the nitrate is added, care must be taken to avoid spattering.)

TABLE 4.

Determinatin of Magnesium

(Mg. precipitated as the crystalline $MgHPO_4$ from solutions containing alkalies and excess phosphates by gradually advancing the *p*H from 6.7–6.8 with dilute ammonia.)

THEORY *mg MgO*	FOUND *mg MgO*
100	100.77
100	100.00
100	99.25
100	99.10

The associate referee finally attempted to determine whether iron and aluminum, when present in quantities larger than those suitable for colorimetric determinations, could be determined quantitatively as the phosphate when precipitated under the conditions given for their removal in the directions sent to the collaborators. According to Patten[1] ferric phosphate is precipitated at a *p*H of 2.0, while precipitation of aluminum is not completed until the *p*H almost reaches 4.0. In his experiments the associate referee made all precipitations at this last *p*H. He suggests,

[1] *This Journal*, 6, 418 (1923).

however, that if the *p*H were carefully controlled by precipitating the iron and aluminum from a solution containing excess phosphate by a weak solution of alkaline buffer, such as a 2–5 per cent solution of sodium or ammonium acetate in the presence of an indicator such as thymol blue (acid range), it might be possible to separate the iron from the aluminum. This suggestion is made for the benefit of the next referee. Solutions containing known quantities of iron and aluminum were prepared from pure iron wire and aluminum foil. The associate referee made numerous experiments on the precipitation of aluminum (as the phosphate) and several on

TABLE 5.

Results of the determination of iron and aluminum as the phosphates.
(Washing made with cold water)

THEORY	SALTS PRESENT	1ST PRECIPITATION		2ND PRECIPITATION	
mg.		*mg.*	*per cent*	*mg.*	*per cent*
Al_2O_3					
5.55	NaCl	—	—	5.64	101.6
18.5	Na Acetate	—	—	19.37	104.0
37.0	Mg, Ca, K salts	—	—	36.4	98.0
21.57	Mn Salts	—	—	21.59	100.0
6.47	Mg, Ca, K and Mn salts in large amounts	—	—	6.57	101.0
25.49	Mg, Ca, K and Mn salts in large amounts	32.38	127.0	24.60	97.0
50.98	Mg, Ca, K and Mn salts in large amounts	62.26	122.0	50.79	99.6
Fe_2O_3					
29.0	Sodium Salts	—	—	31.38	108.0
29.0	Sodium Salts	—	—	30.53	104.2
29.0	Ammonium Salts	—	—	28.20	97.0
29.0	Ammonium Salts	—	—	29.89	103.0
29.0	Ammonium Salts	—	—	29.91	103.0
29.0	Mn, Mg, Ca, K Salts	—	—	29.42	101.3
14.5	Mn, Mg, Ca, K Salts	—	—	14.81	102.0

iron, the details of which it is probably unnecessary to describe. He did find, however, that the temperature of the washing water and the quantity of the electrolytes, particularly alkali salts, influenced the results. If the precipitate is washed with hot water, especially if the electrolytes are low, both iron and aluminum phosphates tend to hydrolyze, breaking down partially into oxides and phosphoric acid, the latter being lost. This is especially noticeable in the case of the ferric phosphate, the ignited precipitate being often a decided brown. The associate referee found that hydrolysis was much reduced if the hot solution after the precipitation of the phosphates was cooled to room temperature and then filtered and washed with *cold* water. If the phosphates were precipitated once from solutions containing alkali electrolytes in quantity the results were apt

to be high. The associate referee therefore dissolved the first precipitate and then precipitated the phosphates a second time by adjusting the *p*H to 4.0 with dilute ammonia and ammonium acetate. All washings were made in the cold. It was found that filter pulp added to the solution previous to the last precipitation assisted in the rapid washing and ignition of the phosphates. Proceeding in this way he obtained quite satisfactory results although the determination of iron and aluminum phosphates in this manner seems to be influenced to some extent by a compensation of errors. Both phosphates are very gelatinous and difficult to wash. As soon as the electrolytes present become low in concentration in the precipitate on washing more or less hydrolysis sets in, varying with the temperature. The road to success therefore is believed to lie in a reprecipitation of the phosphates from solutions containing no non-volatile precipitants and washing with cold washing solutions containing volatile electrolytes adjusted to a *p*H of perhaps 4.0. Under such conditions both contamination and hydrolysis should be reduced. Some of the results obtained are shown in Table 5. These results are suggestive and seem promising for future development.

SUMMARY

1. Iron and aluminum appear to be quantitatively separated from the other plant bases by precipitation as the normal phosphates at a *p*H of 4.

2. Collaborative results are quite satisfactory. The analysts with more experience with these methods seemed to have more success than the inexperienced analysts.

3. The single precipitation method for the determination of calcium yields results as accurate as those obtained by the double precipitation methods.

4. Magnesium results by the method sent to collaborators are usually slightly high.

RECOMMENDATIONS[1]

It is recommended—

(1) That the recommended method for the determination of potassium be changed by inserting the words "on a tared Gooch crucible, with an asbestos mat which has been washed thoroughly with 90 per cent alcohol and dried at 100°C." between the words "Filter" and "wash the precipitate thoroughly with 90 per cent alcohol."

(2) That in the reagents for the determination of manganese, calcium and magnesium reagent (c) be changed to 3 per cent oxalic acid solution and the word "saturated" in the single calcium precipitation method be removed.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 64 (1931).

(3) That the magnesium method using crystalline precipitation replace the method submitted to collaborators.

(4) That new work include collaborative effort on the determination of iron and aluminum.

(5) That the proposed methods be included as tentative methods in *Methods of Analysis*

The writer wishes to take this opportunity to express appreciation to the referee for his assistance in this work and in the writing of this report.

No report on canned foods was given by the referee.

The address of the president, E. M. Bailey, was published on p. 18 of Vol. 14, *This Journal.*

SECOND DAY

TUESDAY—AFTERNOON SESSION

REPORT ON CEREAL PRODUCTS

By J. A. LeClerc (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

During the year ended today, considerable collaborative and investigational work has been conducted by the various associate referees on cereal products and to these workers belongs the credit for the progress that has been made since the last meeting.

METHODS OF SAMPLING FLOUR

Associate Referee H. Runkel reported on methods of sampling flour to establish their accuracy with respect to other determinations than moisture. Pairs of collaborators were requested to sample individually the same pile of flour according to the procedure already adopted. Twelve composite samples of flour were thus obtained. These were analyzed for moisture, nitrogen and ash. The results of analysis are very satisfactory. This method of sampling has been given a most thorough study (1) with respect to the accuracy of sampling a single sack; (2) with respect to the accuracy by which two samplers check each other on the same pile of flour; and (3) regarding the detail of making composite samples of satisfactory uniformity as regards composition.

GLUTENIN

Associate Referee M. J. Blish, made a special study of the influence of the *p*H, kind of salts, concentration of salts, kind of alcohol (methyl or ethyl), concentration of alcohol and temperatures on both the amount and composition of the non-gliadin protein precipitated from acetic acid alcohol dispersions. As a result of these studies the associate referee has serious doubts as to the validity of the generally accepted belief that wheat gluten is composed essentially of two distinct, individual proteins, gliadin and glutenin.

STARCH IN FLOUR

Associate Referee L. H. Bailey conducted collaborative studies with the so-called Rask method, now tentative, and with the Hartmann and Hillig modification of the diastase method. The Rask method has given more satisfactory results than has the modification of the diastase method. These studies have resulted in a further improvement of the Hartmann and Hillig method by the authors themselves. Much is expected from this newer modification.

FLOUR-BLEACHING CHEMICALS

Associate Referee G. C. Spencer used the Seidenberg method of determining chlorine in chlorine-bleached flour to conduct collaborative experiments. As a result of this work it is proposed to test a modification of this method consisting of the addition of ammonium or sodium bicarbonate to the ether extract before evaporating to dryness so as to obviate any reaction with the platinum of the dish.

METHODS FOR SAMPLING AND DETERMINATION OF MOISTURE IN BREAD

Associate Referee L. H. Bailey carried on collaborative work to determine the possibility of utilizing only one-half of the loaf of bread instead of the whole loaf in preparing a sample of bread for the determination of moisture, nitrogen, ash, etc. For this purpose the following types of bread were used: rye, whole wheat, Vienna, and sandwich. Four collaborators assisted in this work, and in each half of the loaf of bread the moisture, ash, and nitrogen were determined. The results indicate that it is perfectly feasible to utilize one-half of the loaf for purposes of chemical examination. This work fully corroborates that of last year.

LIPOIDS AND FAT IN BAKED PRODUCTS

Associate Referee J. H. Bornmann studied the methods for both fats and lipoids in alimentary paste and bread. Three samples—egg noodles, macaroni, and white bread—were submitted to each of three collaborators. The results for fat, lipoids, and lipoid phosphoric acid are quite satisfactory. They are summarized as follows:

	EGG NOODLES			MACARONI			BREAD
	Fat	*Lipoids*	*Lipoid* P_2O_5	*Fat*	*Lipoids*	*Lipoid* P_2O_5	*Fat*
Min.	4.78	5.06	.10	1.68	1.87	.031	6.87
Max.	5.03	5.39	.12	1.83	2.07	.040	7.09
Av.	4.91	5.23	.11	1.72	1.99	.037	6.96

A comparative study of the official method for the determination of lipoids and of a modification thereof proposed by L. C. Mitchell, namely, using alcohol-chloroform mixture instead of an alcohol-ether mixture, was made on these samples. The results show that in each case a higher content of lipoids is obtained by the Mitchell modification, amounting in the case of egg noodles to 0.5 per cent, in the case of macaroni to 0.2 per cent, and in the case of bread, to 1.09 per cent. The amount of phosphoric acid in each one of these products analyzed by the Mitchell modification was likewise greater by about 0.02 per cent than by the official method.

ORGANIC AND AMMONIACAL NITROGEN IN AIR-DRIED BAKED CEREAL PRODUCTS

Studies were carried on by Associate Referee S. C. Rowe to determine the applicability to baked products of the official method for the determination of organic and ammoniacal nitrogen in flour. For that purpose

two samples of bread and two of cake were sliced, air dried, ground to pass a 20-mesh sieve, and analyzed for nitrogen by the three official methods, namely, the Kjeldahl, the Gunning, and the Kjeldahl-Gunning-Arnold. In each case the Kjeldahl method gave results from 0.03 to 0.08 per cent lower than those obtained by the Gunning or by the Kjeldahl-Gunning-Arnold tests. The results from the Gunning and from the Kjeldahl-Gunning-Arnold methods agree very closely.

CRUDE FIBER IN ALIMENTARY PASTES AND IN AIR-DRIED BAKED CEREAL PRODUCTS

The method for determining crude fiber in these products was studied by Associate Referee R. L. Horst. Two samples of spaghetti and three samples of bread were subjected to collaborative tests with the following results:

	Min	*Max*	
Whole Wheat Bread	1.62	2.24	3 collaborators
Graham Bread	0.86	1.70	
White Bread	0.28	0.78	
Spaghetti No. 1	0.22	0.51	4 collaborators
Spaghetti No. 2	0.23	0.53	

EXPERIMENTAL BAKING TESTS

Associate Referee M. J. Blish has again acted as a referee for both the A.O.A.C. and the A.A.C.C., on the subject of a standard laboratory baking test. The work of the collaborators is regarded as reasonably satisfactory and encouraging and as a result the associate referee reports that this test appears now to be definitely established on sound principles. Certain factors, however, especially the method and degree of mixing, molding and baking, still require intensive study.

UNSAPONIFIABLE MATTER IN FLOUR AND IN ALIMENTARY PASTES AND WATER-SOLUBLE PROTEIN IN ALIMENTARY PASTES

After considerable research, Associate Referee Samuel Alfend is of the opinion that further collaborative work on the method for the determination of unsaponifiable matter in the fat of flour and cereal products would prove unprofitable.

This associate referee has further studied the method for determining the crude albumin nitrogen in alimentary pastes and obtained better results by the indirect method, in which the nitrogen is determined before and after precipitation with corresponding saving of time and with no loss in accuracy.

COLLECTING AND PREPARING SAMPLES OF ALIMENTARY PASTES FOR ANALYSIS

Associate Referee Rowe studied the tentative method of collecting and preparing samples of alimentary pastes for analysis. This method requires

that the macaroni shall be quantitatively weighed before and after grinding if the total solids of the original unground material is desired. In this study it was found that as a result of grinding the sample and sifting it through a 20-mesh sieve, a loss amounting to 0.2 per cent took place. The associate referee maintains that this loss is partly mechanical and should not be credited solely to the loss of moisture. He is of the opinion that by grinding a sample no finer than here indicated, very little if any moisture is lost.

MOISTURE IN ALIMENTARY PASTES

The tentative method for the determination of moisture in macaroni products and in baked products has again been studied by Associate Referee Rowe. Two samples each of macaroni, bread, and cake were analyzed for moisture by drying for one hour at 130° and for five hours by the vacuum method at 98° with a pressure of less than 25 mm. Closely agreeing results by these two methods were obtained in the case of both macaroni and bread. With cake, however, the air oven gave results from 0.25 to 0.30 per cent higher than those obtained by the vacuum method.

RECOMMENDATIONS[1]

Flour

It is recommended—

(1) That the tentative method (official, 1st action) of sampling flour be made official, final action, and that further collaborative work be discontinued for the present.

(2) That until the chief protein constituents of wheat flour are reliably identified and correlated with flour properties or characteristics, attempts to develop methods for the quantitative determination of glutenin be discontinued.

(3) That further collaborative work be carried on with the tentative (Rask) method of determining starch, and that it be compared with the more recent modification of the Hartmann and Hillig method.

(4) That the tentative Seidenberg method for determining chlorine in chlorine-bleached flour be again studied and compared with a modification thereof which consists of adding ammonium or sodium bicarbonate to the ether extract, and that studies be made for the detection of benzoic acid in flours treated with organic peroxides.

(5) That special (non-collaborative) studies be continued on the methods for unsaponifiable matter in flour, alimentary pastes and baked products in conjunction with the same study on eggs.

(6) That further study of rapid methods of ashing flour, bread, and other baked products be made.

(7) That a comparative study be made of the colorimetric and quinhydrone-electrode methods of determining the hydrogen-ion concentra-

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 65 (1931).

tion in order to determine the applicability of the colorimetric method to cereal products.

(8) That studies be made of methods of estimating the diastatic value of flour.

(9) That comparative tests be made of foreign and domestic methods of chemical analysis used as a measure of determining the value of flour.

(10) That the present tentative method for water-soluble protein nitrogen precipitable by 40 per cent alcohol be dropped and that in its stead the proposed tentative method for "Albumin Nitrogen" be adopted.

(11) That methods for the determination of CO_2 in self-rising flour be studied.

Alimentary Pastes

(1) That the tentative method for the determination of moisture in alimentary pastes be further studied.

(2) That the method adopted officially (1st action) for the determination of crude fiber in flour be further studied to determine its applicability to alimentary pastes and bread.

(3) That the tentative (official, 1st action) method for the determination of fat by acid hydrolysis in alimentary pastes be further studied.

(4) That the tentative (official, 1st action) method for the determination of lipoids and lipoid phosphoric acid in alimentary pastes be studied collaboratively in comparison with the chloroform-alcohol extraction method suggested by Mitchell.

(5) That the present tentative method of collecting and preparing a sample of alimentary paste for analysis be dropped and that the method given in the referee's report be substituted in place thereof:

Bread and Baked Products

(1) That a study be made of methods to determine milk solids in bread.

(2) That a study be made of methods to determine rye in bread.

(3) That the method (official, 1st action) to determine chlorides in baked products be studied for the purpose of making this method official, final action.

(4) That the tentative standard baking test be continued as such.

(5) That the tentative method for the determination of moisture in bread be made official, final action.

(6) That the tentative method (official, 1st action) for the determination of moisture in cake be further studied.

(7) That the method for total solids in air-dried bread by heating at 130°C. for one hour be further studied.

(8) That for the purpose of making the usual chemical determinations in bread one half of the loaf be taken as a sample. (Official, 1st action).

(9) That the tentative method for determining fat in bread by acid hydrolysis be made official, 1st action.

(10) That further study be made of the method to determine lipoids in baked products.

REPORT ON SAMPLING OF FLOUR

By H. RUNKEL (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

In accordance with last year's recommendation, collaborative study was undertaken to secure additional data on the accuracy of the method with respect to determinations other than moisture. In addition, study was made of the detail of combining all cores into one container instead of using a container for each sack sampled.

*Analysis of collaborative flour samples**

(To show the accuracy with which one sampler may check another on the same pile of flour by the use of the sampling method by combining the cores. Also to show the accuracy with respect to determinations other than moisture.)

DESCRIPTION	SAMPLER	MOISTURE (1)	ASH (1)	NITROGEN (2)
		per cent	*per cent*	*per cent*
400-140 lb. sacks, piled 3 x 11 x 11,	RCJ	13.59	0.49	1.56
stored 27 days at St. Louis, Mo.	JAP	13.56	0.48	1.61
	(Diff.)	0.03	0.01	0.05
576—98 lb. sacks, piled 9 x 13 x 5,	WBS	12.98	0.38	1.35
stored 30 days at Chicago, Ill. 1-inch	WBT	12.98	0.37	1.43
trier used	(Diff.)	0.00	0.01	0.08
400—98 lb. sacks, piled 9 x 10 x 8,	GHE	13.43	0.48	2.12
stored 8 days at New Orleans, La.	ECD	13.48	0.48	2.01
	(Diff.)	0.05	0.00	0.11
50—48 lb. sacks, piled 5 high, stored 21	MOB	13.03	0.40	1.88
days at Kansas City, Mo. Mixed on	TBB	13.21	0.41	1.94
paper before delivered to container	(Diff.)	0.18	0.01	0.06
159—48 lb. sacks, piled 8 x 4 x 5,	MOB	13.16	0.39	1.91
stored 21 days at Kansas City, Mo.	TBB	13.09	0.39	1.94
Mixed on paper before delivered to container	(Diff.)	0.07	0.00	0.03
120—48 lb. sacks, piled 12 x 5 x 2,	MOB	13.24	0.49	1.93
stored 14 days at Kansas City, Mo.	TBB	13.24	0.49	1.94
Mixed on paper before delivered to container	(Diff.)	0.00	0.00	0.01

* Average of triplicate determinations.
(1) Analyses by I. S. Shupe; (2) T. C. Dunn, checked by H. R. Bond.
Samples were drawn by R. C. Jordan and J. A. Pitts; W. B. Simmons and W. B. Tiedt; G. H. Eigenberger and E. C. Deal; and M. O. Bourne and T. B. Benjamin.

Collaborators were asked to work in pairs and to sample individually the same pile of flour according to the method, avoiding each other's selection of sacks. Each collaborator was asked to combine the cores into one composite sample. Analysts in the Chicago Station reported the results given in the table.

It will be noted that reasonable checks were obtained on moisture, ash, and nitrogen. The differences between pairs demonstrate the accuracy of the method of sampling. These differences approximate those found in previous collaborative work, and are favorably comparable with analytical variations. It is apparent that the method is about equally accurate for other determinations than moisture.

No apparent difficulty in combining the cores was noted except that when a one-inch trier was used the sample was too large for one container and was difficult to mix properly. Accordingly, the ½-inch trier mentioned in the method is believed more desirable. The analytical results on the composite samples appear to be satisfactory.

The method has now been studied collaboratively—

First, with respect to the principles, see *This Journal*, **9**, 423 (1926), and **10**, 450 (1927), where report is made on the comments received from various trade representatives and representatives of the American Association of Cereal Chemists, American Institute of Baking, and Millers' National Federation, as well as numerous food officials;

Second, with respect to accuracy of the sampling of a single sack by the method, see *This Journal*, **10**, 450 (1927), where the procedure outlined appears to be satisfactory;

Third, with respect to the accuracy by which two samplers check each other on the same pile of flour, see *This Journal*, **10**, 450 (1927) and **11**, 464 (1928), and the present report, where it is apparent that the method gives results, the variations of which are comparable to analytical variations; and

Fourth, with respect to the detail of combining the sample as well as the applicability of the method to other determinations than moisture (present report) where it appears that the cores may be combined into one composite sample and the method applied to determinations other than moisture without impairing its usefulness and accuracy if there is no question as to shrinkage or uniformity of the lot.

It is not apparent that further collaborative work is necessary on this method to demonstrate its value.

The comments received from time to time have been given careful consideration and have been very helpful. The detail which was discussed most frequently concerned combining the cores into a composite sample. The method, as outlined, is applicable when a sample is drawn for moisture in connection with the estimation of shrinkage where net weight de-

terminations are involved. It is also applicable if the uniformity of the lot is questioned. The method is applicable to the drawing of samples for such determinations as are listed in *Methods of Analysis*. If shrinkage is not involved and the uniformity of the lot is not questioned, the work this year indicates that the cores may be combined into a composite sample, if this procedure suits the purpose for which the sample was drawn. While this procedure is suggested in the last paragraph, the comments received and a study of the work of the collaborators indicate that users of the method may be somewhat confused as to when to combine and when not to combine cores. Accordingly it is recommended that the following explanatory paragraph be inserted before the last paragraph:

For determinations mentioned in *Methods of Analysis*, where shrinkage or uniformity is not involved, all cores may be delivered into one of the above containers of such size that the composite sample will fill it not more than three-fourths full.

Since the method with this explanatory statement appears to have as general application as it seems possible to develop at this time and its present status is official (first reading), it is recommended that the statement be added and the method adopted as official, final action.[1]

No report on ash in flour and gasoline color value was given by the associate referee.

REPORT ON GLUTENIN IN FLOUR

By M. J. Blish (Agricultural Experiment Station, Lincoln, Nebr.), *Associate Referee*

Last year's report emphasized the viewpoint that glutenin, as prepared, described and characterized by Osborne,[2] can no longer be regarded as a distinct protein of established individuality. Blish and Sandstedt[3] showed that both the amount and composition of "glutenin" as prepared by the conventional procedure (involving the use of alkali as a dispersing agent) will vary according to the strength of the alkali or extent of exposure thereto, and that there is probably some irreversible degradation of a complex protein substance regardless of the concentration of the alkali. They undertook to isolate glutenin by a procedure in which exposure to an alkaline reaction would be strictly avoided at all stages. It was found that a dispersion of gluten in very dilute acetic acid in the presence of 50–60 per cent alcohol can be made to serve as a starting point for the separation of the gliadin and non-gliadin constituents of the gluten. It seems reasonable to assume a minimum of alteration in the chemical constitution and identity of the original protein mass when dispersed in such a medium.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 65 (1931).
[2] The Proteins of the Wheat Kernel, Carnegie Inst. of Washington, 1907.
[3] *J. Biol. Chem.*, 85, 195 (1929).

From dispersions of this character it is possible to coagulate and precipitate large portions of protein either by neutralization or by the addition of small quantities of neutral salts, while maintaining an alcoholic concentration that is presumably sufficient to retain all the gliadin in solution. Serious difficulties are encountered, however, in attempting to establish the true character of the precipitated protein. It varies, both in amount and composition, in accordance with variations in the conditions under which it is precipitated.

During the past year the work of the associate referee has been concerned chiefly with studies of individual factors, respectively, as influencing both amount and composition of the non-gliadin protein precipitated from acetic acid-alcohol dispersions. Among the factors subjected to individual study were *p*H, kind of salt, concentration of salt, kind of alcohol (methyl vs. ethyl), concentration of alcohol, and temperature. For present purposes it is sufficient to say that certain of these factors have an enormous influence upon the character of the precipitated protein. For example, K_2SO_4 precipitates far more protein than NaCl, other things being equal. All salts in 0.1 *N* concentration precipitated more protein than in 0.05 *N* concentration. The temperature factor is important, and under some conditions very critical. Thus, in the neighborhood of room temperature, a few degrees may mean the difference between a heavy, sharply defined precipitation and no precipitation at all. When ethyl alcohol is used for the purpose of retaining the gliadin in solution, much lower temperatures are necessary to secure precipitations equal in amount to those obtained under similar conditions with methyl alcohol.

In the light of these findings, the associate referee entertains serious doubts as to the validity of the generally accepted belief that wheat gluten is composed essentially of a mixture of two distinct individual proteins, gliadin and "glutenin." By slight variations of chemical or physical treatment it is possible to prepare several protein "fractions" differing from each other both in chemical and physical properties. With the possible exception of gliadin, the proteins of wheat flour have not yet been reliably identified and characterized, and the problem requires much further investigation. The associate referee recommends[1] that until these constituents are reliably identified and correlated in some useful manner with flour characteristics or properties, attempts to establish methods for their quantitative estimation be discontinued.

No report on the hydrogen-ion concentration of flour was given by the associate referee.

No report was given on diastatic value of flour as no associate referee on this subject was appointed.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 65 (1931).

REPORT ON STARCH IN FLOUR

By L. H. BAILEY (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

Following the directions of Subcommittee C, work was done on the determination of starch in flour, bread and alimentary paste by comparing the results obtained by the tentative method given in *This Journal*, 11, 37 (1928) with results obtained by a modification of the method proposed by Hartmann and Hillig for determining total carbohydrates in cereal products (*This Journal*, 9, 482 (1926)). Collaborative results were obtained on the flour. The associate referee also applied the two methods to bread and macaroni.

With all the products more satisfactory results were obtained with the tentative method than with the modified method. During the course of the work, however, Hartmann and Hillig made some decided changes in their method and now have developed a method that completely converts the starch to sugar. They have obtained very satisfactory results with this method. It has been published.[1]

In view of the development of this new method a detailed report of work with the modified method is omitted at this time.

It is recommended[2] that next year this new method be studied and applied to the determination of starch in cereal products in comparison with the present tentative method of determining starch in flour.

REPORT ON FLOUR-BLEACHING CHEMICALS

By G. C. SPENCER (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

Attention was given this year to the possible tests for the presence of benzoyl peroxide in flour that has been bleached by this chemical. So far as could be learned, the benzoyl peroxide seems to be entirely destroyed by action of flour within 24 hours after the two are intimately mixed.

The method of S. Rothenfusser[3] is a delicate test for traces of benzoyl peroxide. This method was modified in the New York Station of the Food and Drug Administration and consequently simplified. The only recourse seems to be a method for detecting the minute quantities of benzoic acid that are left in the flour by the decomposition of the peroxide.

The Seidenberg[4] method for estimating added chlorine in chlorine-bleached flour was given further trial by collaborative workers this year; the results appear in the accompanying table. The samples used repre-

[1] *This Journal*, 14, 112 (1931).
[2] For report of Subcommittee C and action of the association, see *This Journal*, 14, 65 (1931).
[3] *Chem. Ztg.*, 39, 285 (1925).
[4] *This Journal*, 11, 132 (1928).

sented the same types of bleaching that were employed a year ago, viz: (a) An *Agene* bleach and (b) a *Beta-Chlora* bleach.

Chlorine in bleached flour.
(Results expressed in parts per million, water-free basis.)

COLLABORATOR		1	2	MAX	MIN.	AVERAGE
L. H. Bailey	(a)	10.0				10.0
	(b)	131.4				131.4
F. B. Carpenter	(a)	24.9	18.3			21.6
	(b)	150.0	142.0			146.0
W. C. Luckow	(a)			10.9	0	2.8†
	(b)			154.8	138.5	144.1†
Dorothy B. Scott	(a)	0	0			0
	(b)	125.0	116.0			120.5
				Grand Averages	(a)	3.0
					(b)	135.5

* Average of 8 results
† Average of 10 results

The associate referee suggests a modification of the original method which it is proposed to incorporate into next year's procedure. It has been noted that when the alkaline concentrate of the ether extracts is charred in a platinum dish there is sometimes enough caustic alkali present to react with the platinum in spite of careful heating. This can be obviated by adding to the extract before evaporating to dryness and charring a sufficient quantity of ammonium or sodium bicarbonate solution. Preliminary trials indicate that this may be a practical modification.

RECOMMENDATIONS[1]

It is recommended—

(1) That work on the Seidenberg method for determining added chlorine in bleached flour be continued.

(2) That the method be modified by the addition of ammonium or sodium bicarbonate, as indicated in this report.

(3) That efforts be continued toward the detection of benzoic acid in flours bleached with organic peroxides.

No report on foreign methods for testing flour was given by the associate referee.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 65 (1931).

REPORT ON SAMPLING AND DETERMINATION OF MOISTURE IN BREAD

By L. H. BAILEY (U. S. Bureau of Chemistry and Soils, Washington, D. C.), *Associate Referee*

In 1929 Subcommittee C recommended (1) that collaborative study be made of the tentative method for sampling bread to determine the possibility of utilizing only one-half of the loaf instead of the whole loaf and (2) that different types of bread be tried.

In pursuance of this recommendation duplicate loaves were secured of each of the four following types of bread: rye, whole wheat, Vienna and sandwich. Four collaborators were secured to make this study. Each collaborator was given duplicate loaves of one kind of bread and asked to make the following determinations: moisture, ash, and nitrogen. One loaf was to be treated as one sample, but the duplicate loaf was to be cut in half and each half treated as a separate sample. The analytical results were supposed to show whether or not the composition of the half loaves was the same as that of the whole loaves.

The analytical results follow. The moisture was determined on the fresh loaf and the ash and nitrogen on the air-dried samples in each case.

The analyses indicate that the composition of either half of a loaf of bread is practically the same as that of the whole loaf.

In the work last year the loaves were quartered, and moisture was determined in each quarter as in a whole loaf. Four types of bread were included in this study. It was pointed out that the moisture content varies in the different quarters from 0.72 per cent to 1.43 per cent, which showed that it would not be permissible to use a quarter of a loaf to get a representative sample. Further study of these results, however, brought out the fact that if half loaves were considered instead of quarters the results were practically the same as with the whole loaves. Thus by averaging the moisture results of the adjacent quarters forming the ends of the loaves,

Whole loaves vs. half loaves
(Results expressed in percentage)

	RYE BREAD			WHOLE WHEAT BREAD			SANDWICH BREAD			VIENNA BREAD		
	WHOLE LOAF	DUPLICATE LOAF		WHOLE LOAF	DUPLICATE LOAF		WHOLE LOAF	DUPLICATE LOAF		WHOLE LOAF	DUPLICATE LOAF	
		1ST HALF	2ND HALF		1ST HALF	2ND HALF		1ST HALF	2ND HALF		1ST HALF	2ND HALF
Moisture (fresh basis)	35.27	34.40	34.17	35.18	34.00	33.95	37.28	37.38	37.46	33.58	33.28	33.68
Ash (air-dried basis)	2.53	2.51	2.58	2.97	2.91	2.92	2.52	2.49	2.52	2.06	2.10	2.12
Nitrogen (air-dried basis)	1.93	1.92	1.95	1.95	1.93	1.90	1.96	1.99	1.99	1.99	1.96	1.99

the differences in moisture between the halves of the four loaves varied only from 0.04 per cent to 0.19 per cent, and maximum differences in moisture between either end and the moisture in the whole loaf varied from 0.02 per cent to 0.14 per cent.

Since the results obtained last year when half loaves were taken as samples, as determined by one operator, and the results obtained again this year by four operators with half loaves as samples showed that the composition of a half loaf is practically the same as a whole loaf it is suggested that not less than half a loaf of bread be taken as a sample for analysis.

The rye bread was analyzed by Mayne R. Coe, U. S. Bureau of Chemistry and Soils, the sandwich bread by G. Smith, U. S. Food and Drug Administration, the whole wheat bread by V. E. Munsey, U. S. Food and Drug Administration, and the Vienna bread by L. H. Bailey. The associate referee gratefully acknowledges the assistance given by these collaborators.

It is recommended[1] that not less than half a loaf of bread be taken as a sample for analysis.

REPORT ON LIPOIDS AND FAT IN ALIMENTARY PASTES AND IN BAKED PRODUCTS

By J. H. BORNMANN (U. S. Food and Drug Administration, Chicago, Ill.), *Associate Referee*

In accordance with last year's recommendation of Subcommittee C, a study of the methods for determining fat by acid hydrolysis, lipoids and

TABLE 1.

Collaborative results on lipoids, lipoid phosphoric acid (P_2O_5), and fat by acid hydrolysis in bread and in alimentary paste.

COLLABORATOR	EGG NOODLES FAT	EGG NOODLES LIPOIDS	EGG NOODLES LIPOID P_2O_5	MACARONI FAT	MACARONI LIPOIDS	MACARONI LIPOID P_2O_5	BREAD FAT
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
V. E. Munsey	4.78	5.38	0.11	1.68	1.87	0.038	6.96
Washington	4.80	5.39	0.12	1.74	1.96	0.040	6.99
	4.98			1.83			7.09
I. S. Shupe	5.02	5.06	0.100	1.70	2.07	0.031	6.87
Chicago	5.03	5.09	0.101	1.71	2.05	0.033	6.91
J. H. Bornmann	4.85	5.21	0.110	1.68	1.97	0.039	6.95
	4.90	5.27	0.112	1.68	2.04	0.040	6.96
Maximum	5.03	5.39	0.12	1.83	2.07	0.040	7.09
Minimum	4.78	5.06	0.100	1.68	1.87	0.031	6.87
Average	4.91	5.23	0.109	1.72	1.99	0.037	6.96

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 67 (1931).

lipoid phosphoric acid (P_2O_5) in alimentary pastes and in baked products was undertaken. Three samples were sent to collaborators, namely: egg noodles, macaroni, and white bread. All samples were air dried and were ground to 20-mesh. The methods of analysis submitted with the samples have been published.[1] The collaborative results are given in Table 1.

COMMENTS OF COLLABORATORS

V. E. Munsey.—Methods for the determination of lipoids and lipoid P_2O_5 offered no difficulties and seemed to work all right. The determination of fats by the acid hydrolysis method was done during very hot weather, and it was difficult to work with the ether. I seemed to have trouble in getting the material in solution in the dilute hydrochloric acid and also found that the Röhrig tubes could not be filled as full as the method states, because the hydrochloric acid mixture does not settle sufficiently to drain off the ether, free from contamination. I wonder also if it would be a good idea, after the fat is weighed, to dissolve the fat and weigh the flask again. This procedure would account for the material that is carried over and weighed up as fats.

ALCOHOL-CHLOROFORM EXTRACTION METHOD FOR LIPOIDS

Hertwig[2] showed that the recovery of lipoids from noodles of known composition is not complete. In some recent tests made by the associate-referee, fat was determined by acid hydrolysis on the air-dried residue from the lipoid extraction. These tests showed, respectively, 0.59, 0.42, and 0.56 per cent of fat remaining in the egg noodles, macaroni, and bread. With the hope of finding a method which would give more complete extraction, the alcohol-chloroform extraction method, suggested by L. C. Mitchell, was investigated. It was found that treating alimentary pastes in the cold with a mixture of equal parts of alcohol and chloroform gave low results. Apparently the particles are too dense to be penetrated by the solvent. After some preliminary investigation the method was formulated as follows:

Put 10 grams of the material into an 8-ounce nursing bottle and add 30 cc. of alcohol, 70 per cent by volume. Mix well, stopper, and heat in a water bath at 75°–80°C., with frequent shaking, for 15 minutes. Remove from the bath, add 70 cc. of 95 per cent alcohol, and shake until the liquid appears fairly clear. Cool, add 100 cc. of chloroform, and shake again until the liquid appears fairly clear. Draw off 100 cc. of the supernatant liquid by means of a pipet into a 250 cc. beaker containing some bits of porcelain. From this point the treatment is the same as that given in the tentative method.

Objection may be made to this method on the ground that the product absorbs some of the water and thus changes the volume relation. This point has not yet been investigated, but it appears likely that the 95 per cent alcohol removes the water which may have been absorbed by the sample. On introducing 30 cc. of 70 per cent alcohol, 70 cc. of 95 per cent alcohol, and 100 cc. of chloroform into a 200 cc. volumetric flask there was

[1] *This Journal*, 9, 40 (1926); 11, 38 (1928).
[2] *Ibid.*, 7, 91 (1923).

no change in volume. Any volume change due to dissolved lipoids must be rather small. A test made by this method on alimentary pastes and bread gave some very encouraging results. Lack of time prevented further investigation. Table 2 gives a comparison of results obtained by the tentative method and the alcohol-chloroform extraction method.

TABLE 2.

Comparison of results obtained by tentative method for lipoids and lipoid phosphoric acid (P_2O_5) and by the alcohol-chloroform extraction method.

	EGG NOODLES		MACARONI		BREAD	
	LIPOIDS	LIPOID P_2O_5	LIPOIDS	LIPOID P_2O_5	LIPOIDS	LIPOID P_2O_5
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Official method	5.21	0.110	1.97	0.039	6.53	0.036
	5.27	0.112	2.04	0.040	6.59	0.042
Alcohol-chloroform method	5.77	0.134	2.24	0.055	7.65	0.062

DISCUSSION

Fat

The acid hydrolysis method has been under investigation for some years. Results of collaborators are fairly satisfactory, both on alimentary pastes and on bread. The method is better than either the direct extraction method or the alkaline extraction method. Since beginners usually have difficulty owing to the addition of too much alcohol, it is suggested that the directions for adding alcohol be changed to read: "Fill to within 2 cc. of the mark with 95 per cent (by volume) alcohol and cool."

Lipoids and Lipoid Phosphoric Acid (P_2O_5)

The tentative method for lipoids is not fully satisfactory. Good results are obtained by the individual analyst, but agreement among collaborators is not satisfactory. As shown by the tests made on the residue from lipoid extraction, there is an appreciable amount of fat left in the material. Incidentally, the extraction appears to be as complete for bread as for alimentary paste, which was unexpected, because it was thought that the lipoids were more firmly enclosed in a baked product. It is hoped that a method may be developed which will insure a more complete extraction than is possible with the present tentative method.

RECOMMENDATIONS[1]

It is recommended—

(1) That the tentative method (official, first action) for determining fat by acid hydrolysis in alimentary pastes be made official (final action).

(2) That the tentative methods (official, first action) for determining lipoids and lipoid phosphoric acid (P_2O_5) in alimentary pastes be studied

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 67 (1931).

collaboratively in comparison with the chloroform-alcohol extraction method suggested by Mitchell.

(3) That the tentative method for the determination of fat by acid hydrolysis in baked cereal products be made official (first action).

(4) That further study be made of methods of determining lipoids in baked products.

No report on milk solids in milk bread was given by the associate referee.

REPORT ON ORGANIC AND AMMONIACAL NITROGEN IN AIR-DRIED BAKED CEREAL PRODUCTS

By S. C. Rowe (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In accordance with the recommendation approved by Committee C, Analysts J. T. Keister of the Food and Drug Administration and O. F. Krumboltz of the Bureau of Chemistry and Soils collaborated with the associate referee in a study of the tentative method for the determination of organic and ammoniacal nitrogen in baked products.[1] The method calls for a determination of nitrogen by any one of three methods, namely, the Kjeldahl, the Gunning, and the Kjeldahl-Gunning-Arnold. Two different samples of bread and two different samples of cake were prepared in the usual manner, i.e., slicing, air drying and grinding to pass a 20-mesh sieve. In connection with the samples of cake it may be pointed out that the air drying should be performed by placing the sliced cake upon glass or some non-absorbing material for the reason that ordinary paper absorbs the fat. Each sample was run for nitrogen by the three methods with the following results:

METHOD	BREAD NO. 1 *per cent*	BREAD NO. 2 *per cent*	CAKE NO. 1 *per cent*	CAKE NO. 2 *per cent*
Kjeldahl	2.12	1.99	0.95	0.91
Gunning	2.16	2.07	0.98	0.98
Kjeldahl-Gunning-Arnold	2.16	2.05	0.99	0.94

It will be noted that the Kjeldahl method gives consistently lower results than do the other two methods. The differences, however, are not great, being a maximum of 0.08 per cent in the case of Bread No. 2 and a minimum of 0.03 per cent with both samples of cake.[2]

[1] *Methods of Analysis, A.O.A.C.*, 1925, 232.

[2] For report of Subcommittee C and action of the association, see *This Journal*, 14, 66 (1931).

REPORT ON CRUDE FIBER IN ALIMENTARY PASTES AND IN AIR-DRIED BAKED CEREAL PRODUCTS

By R. L. HORST (U. S. Food and Drug Administration, New Orleans, La.), *Associate Referee*

The samples were prepared in accordance with the official methods as recommended for study and sent out to six collaborators. Two of these men made the determination on all the samples, and one man completed three of them. The products were graham bread, whole wheat bread, white bread and two semolina spaghetti from different manufacturers.

Samuel Alfend, St. Louis Station, reported:

Spaghetti No. 1	0.36, 0.38, and 0.39%
Spaghetti No. 2	0.40, 0.40, and 0.46%
White bread	0.46, 0.46, and 0.45%

He commented as follows:

Two difficulties were encountered—lumping and slow filtration. The lumping was so severe, even in the acid digestion, that several determinations were lost, and I had to add glass beads to the rest. Because of slow filtration it was necessary to filter the alkaline solution through linen and then to transfer the residue to a Gooch crucible. Although the checks are not extremely bad I have no great faith in the results.

H. P. Strack, Tennessee State Chemist, Nashville, Tenn., reported the following results of one of the men in his laboratory, Charles Buchwald:

Whole wheat bread	1.98, 1.95, 2.12, 2.24, 1.88, 1.93%
Graham bread	1.34, 1.30, 1.70, 1.55, 1.48, 1.33%
White bread	0.51, 0.62, 0.65, 0.78, 0.62, 0.49%
Spaghetti No. 1	0.51, 0.32, 0.51, 0.43, 0.42%
Spaghetti No. 2	0.44, 0.44, 0.43, 0.49, 0.53%

He made no comments on the method.

V. E. Munsey of the Food and Drug Administration, Washington, D. C. reported:

Whole wheat bread	1.83, 1.90%
Graham bread	1.05, 1.04%
White bread	0.34, 0.42%
Spaghetti No. 1	0.25, 0.22%
Spaghetti No. 2	0.24, 0.25%

He commented:

The determination of crude fiber in baked cereal products and alimentary pastes by the regular official method for determining crude fiber seems to work all right and gives results for crude fiber which are probably satisfactory.

G. L. Bidwell of the Food and Drug Administration, Washington, D. C. submitted the following results:

Graham Bread.....1.06, 1.04%
Whole wheat bread....1.82, 1.86%
White Bread.....0.36, 0.40%
Spaghetti No. 1.....0.48, 0.37, 0.50, 0.34, 0.40%
Spaghetti No. 2.....0.45, 0.39, 0.54, 0.36, 0.43, 0.39%

He commented as follows:

I see no reason why the regular official method should not be used for products of this type.

The results of the associate referee are as follows:

Whole wheat bread....1.66, 1.62%
Graham bread.....0.86, 0.91%
White bread.....0.28, 0.36%
Spaghetti No. 1.....0.22%
Spaghetti No. 2.....0.23, 0.30%

DISCUSSION

From the results reported above, it would seem that the method is quite suitable. It may need some changes to make it fully appliable to these products. It would appear that there must have been a difference in some details of the method as carried out by the collaborators.

Undoubtedly the method needs further study and it is recommended that such study be continued the coming year.[1]

No report on rye flour in rye bread was given by the associate referee.

REPORT ON EXPERIMENTAL BAKING TESTS

By M. J. Blish (Agricultural Experiment Station, Lincoln, Neb.), *Associate Referee*

In the development of this project, the past relationship between the Association of Official Agricultural Chemists and the American Association of Cereal Chemists has been maintained. The latter organization has made a few minor changes in the description and specifications of the basic procedure.[2] These minor alterations have been found to be desirable, and the associate referee accordingly recommends that they also be incorporated into the tentative method of the A. O. A. C.[3]

The tentative "standard" laboratory baking test appears now to be definitely established on sound principles. Further improvements will

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 67 (1931).
[2] *Cereal Chem.*, 6, 249 (1929).
[3] *Methods of Analysis, A.O.A.C.*, 1930.

have to do with securing better knowledge and control of those mechanical and environmental factors that are chiefly responsible for a considerable variability wherever the usual type of collaborative testing is undertaken. The more prominent among these factors are concerned with the method and degree of mixing, molding, and baking the dough. In order to undertake a special and intensive investigation of these factors, the American Association of Cereal Chemists has established a Research Fellowship and has accumulated funds sufficient to provide for at least one year's full time work by an experienced technician. During the coming year, starting September 1930, the Research Fellow will work in the Nebraska Agricultural Experiment Station, using the facilities of the Department of Agricultural Chemistry, supplemented by equipment furnished by prominent manufacturers of ovens, molders, mixers, etc.

It is hoped and anticipated that an important outcome of the efforts of the Research Fellow will be the establishment of standard specifications for the mechanical mixing and molding of experimental doughs, to replace the more variable methods involving personal judgment and hand manipulation. Another important subject of proposed investigation by the Fellow is a better understanding of baking oven temperature conditions, and their influence on bread characteristics.

Many wheat and flour testing laboratories, including industrial, governmental, and state institutions are now employing the tentative standard test, at least in so far as amounts and proportions of ingredients and size of baking pans are concerned. One series of collaborative tests, involving four of these laboratories, was conducted during the past year. Each collaborator received two samples of baker's flour, A and B. A was a typical hard winter wheat flour, while B was milled from Early Baart, a popular Pacific coast variety.

The collaborators were requested to make two series of test bakes with each flour, one series using the 3-hour fermentation period specified in the basic procedure, and the other series a 2-hour fermentation, according to Supplementary Method B. All bakes were made in triplicate, with the exception of one collaborator, who baked in duplicate.

The collaborative results were as shown in the following tables. The meaning of the various letters and numerals used for all items in the reports is explained in Cereal Chemistry,[1] and also in last year's report of the associate referee.[2]

All collaborators performed their tests on the same date, and each collaborator immediately mailed a specimen of each series to the associate referee. The volumes of these sample test loaves, respectively, were measured in the same apparatus 6 days after baking. All were smaller than when freshly baked, due of course to shrinkage and drying, but the

[1] *Cereal Chem.*, 6, 249 (1929).
[2] *This Journal*, 13, 458 (1930).

TABLE 1.

Report of collaborator No. 1.

SAMPLE NO.	A	A	A	A	B	B	B	B
Basic Test	—	—			—	—		
Supplementary			B*	B*			B*	B*
Moisture (%)	12.5	—	—	—	11.0		—	—
Flour used (gram)	100.0	—	—	—	100.		—	—
Water used (cc.)	60.5	—	—	—	58.		—	—
Loaf volume (cc.)	525.	530	565	550	510	510	615.	605.
Loaf weight (gram)	132.8	130	134	133	128.2	129.5	130.8	125.2
External	FG	FG	F	FH	F	F	F	F
Internal	4	4	3	3	5	4	3	3
Firmness of crumb	S	S	S	S	M	M	M	M
Texture	M	M	M	M	H	H	S	—
Crust color	M	M	D	D	P	P	D	D
Crust mottling	—	—	—	—	+	+	—	—
Crumb color	Cr	Cr	CR	Cr	Cry	Cr y	Cr	Cr

Remarks

* 2 instead of 3 hours' fermentation

measurements were comparative, since all had presumably undergone the same degree of shrinkage. These measurements were considered desirable in view of the fact that loaf volume measuring devices involving seed displacement give comparative rather than absolute values, unless they have been accurately calibrated and correction factors established. Since one collaborator stated that his apparatus had never been calibrated it was thought that a more reliable indication of concordance among collaborators could be obtained by measuring all samples sent in by the same equipment and under the same conditions. These measurements are recorded in Table 5 which follows.

When considered in the light of previous experiences in collaborative test baking involving different laboratories, varying types of equipment, and varying methods of handling doughs (especially in mixing and molding), these collaborative results may be regarded as reasonably satisfactory and encouraging.

There is fairly good concordance among collaborators in the essential features of the various tests. Agreement among loaf volumes is fairly good throughout, with the exception of Collaborator No. 2, who stated, however, that he used a rather severe mechanical mixing instead of the hand mixing that is specified in the present basic procedure.There is good concordance as to the volume *differentials* between the 2- and 3-hour fermentation periods with both flours. With the exception of Collaborator No. 3, in the case of flour A, all tests showed larger volumes with the 2-hour than with the 3-hour fermentation period. In every instance, flour B, when

TABLE 2.
Report of Collaborator No. 2.

SAMPLE NO.	A	A	A	A	A	A	B	B	B	B	B	B
Basic Test	—	—	—				—	—	—			
Supplementary				B*	B*	B*				B*	B*	B*
Moisture (%)	12.45						11.30					
Flour used (gram)	97.1						95.8					
Water used (cc.)	60.9						58.2					
Loaf volume (cc.)	420.	395.	400.	415.	415.	380.	440.	430.	440.	510.	490.	485.
Loaf weight (gram)	131.	130.5	131.	134.5	135.5	134.5	128.5	127.5	125.5	129.5	130.2	131.2
External	J	J	J	J	J	J	F	F	F	F	F	F
Internal	4	4	4	3	3	3	5		4	3		4
Firmness of crumb	M	M	M	M	M	S	M	M	M	M	M	M
Texture	H	H	H	H	H	H	S	S	S	S	S	S
Crust color	M	M	M	M	MD	M	P	P	P	MD	MD	PM
Crust mottling	—	—	—	—	—	—	+	+	+	+	+	+
Crumb color	Cr Gr	Cr Gr	Cr Gr	Gr	Gr	Gr	Cr W		Cr W	Cr W		Cr W
Remarks	(mixed by machine instead of by hand)							(mixed by machine instead of by hand)				

* 2 instead of 3 hours' fermentation.

TABLE 3.
Report of Collaborator No. 3.

SAMPLE NO.	A	A	A	A	A	A	B	B	B	B	B	B
Basic Test	—	—	—				—	—	—	—		
Supplementary				B*	B*	B*				B*	B*	B*
Moisture (%)	12.87						11.57					
Flour used (gram)	97.6						96.15					
Water used (cc.)	60.4						57.85					
Loaf Volume (cc.)	560	575	545	545	530	520	535	505	510	565	555	560
Loaf Weight (gram)	133	133	132	131	132	130	128	128	127	127	128	126
External	GF	GF	G	i	i	ig	G	Gi	G	F	F	F
Internal	7	7	7	5	5	5	5.5	5.5	5.5	6	6	6
Firmness of crumb	M	M	M	M	M	M	S	S	S	M	M	M
Texture	S	S	S	M	M	M	S	S	S	S	S	S
Crust color	D	D	D	D	D	D	M	M	M	D	D	D
Crust mottling	++	++	++	+	+	+	+	+	+	+	+	+
Crumb color	gr	gr	gr	cr gr	cr gr	cr gr	cr	cr	cr	W	W	W
Remarks												

* 2 instead of 3 hours' fermentation.

TABLE 4.
Report of Collaborator No. 4.

SAMPLE NO.	A	A	A	A	A	A	B	B	B	B	B	B
Basic Test	—	—	—				—	—	—			
Supplementary				B*	B*	B*				B*	B*	B*
Moisture (%)	12.3						11.5					
Flour used (gram)	97.						96.5					
Water used (cc.)	61						57					
Loaf volume (cc.)	464	464	470	502	503	508	458	458	463	510	510	525
Loaf weight (gram)	125.5	125.5	126.9	128	128.4	128.1	125.4	125.9	125.3	123	123	123
External	FJ	FJ	FJ	Fm	Fm	Fm	FJ	FJ	FJ	FG	FG	FG
Internal	4	4	4	3	3	3	4+	4+	4+	6–3	6–3	6–3
Firmness of crumb	M	M	M	M	M	M	S	S	S	M+	M+	M+
Texture	S	S	S	M	M	M	S	S	S	S	S	S
Crust color	D	D	D	D	D	D	P	P	P	D	D	D
Crust mottling	+	+	+	+	+	+	—	—	—	—	—	—
Crumb color	Cr	Cr	Cr	Cr Gr	cr Gr	Cr Gr	wh cr	wh cr	wh cr	wh cr	wh cr	wh cr
Remarks												

* 2 instead of 3 hours' fermentation

TABLE 5.

Loaf volumes 6 days after baking.

SAMPLE	FERMENTATION *hours*	COLLABORATOR 1 *cc.*	2 *cc.*	3 *cc.*	4 *cc.*
A	3	447	345	422	416
A	2	484	372	418	459
B	3	462	380	448	429
B	2	514	445	462	464

handled with the 3-hour period, showed a paler crust color than any of the other test bakes. There were slight, but not serious differences in the baking oven conditions among the collaborators, as indicated by minor variations in crust color. Slight variations among the other loaf characteristics are obviously due to individual peculiarities in molding and panning the dough.

The associate referee recommends[1] that the method be continued as a tentative method and printed in the revised *Methods of Analysis* in the form submitted.

REPORT ON UNSAPONIFIABLE MATTER IN FLOUR AND IN ALIMENTARY PASTES AND WATER-SOLUBLE PROTEIN PRECIPITABLE BY 40 PER CENT ALCOHOL IN ALIMENTARY PASTES

By SAMUEL ALFEND (U. S. Food and Drug Administration, St. Louis, Mo.), *Associate Referee*

The association recommended that special studies be made by the associate referee of the F. A. C. method for the determination of unsaponifiable matter in the fat of flour and of alimentary paste before it was subjected to collaborative study. A thorough check on the details of both the F. A. C. method, which is tentative, and the modified Kerr-Sorber method, which has been dropped, together with several modifications in detail, failed to reveal the cause of the unsatisfactory results obtained by collaborators. The results obtained by the referee were fairly concordant, as they have been in previous years. Individual analysts have been able to obtain fair results, but they have been unable to check each other.

In his report in 1926 the writer suggested that the figure for unsaponifiable matter was of no great value for the purpose for which it had originally been intended, that is, as an indication of the quantity of egg solids present in alimentary pastes. In 1928 the associate referee recommended that no further work be done upon the method.

It is believed that further work on the method as it stands will prove

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 66 (1931).

unprofitable. The same situation exists with respect to this determination in eggs. It is possible that the sterol content of eggs, and the ratio of sterols to unsaponifiable matter, may prove of value in the analysis of egg products. It is felt, therefore, that the study of the unsaponifiable matter in flour and alimentary pastes should be continued, at any rate for another year, in conjunction with work on this method by the Referee on Eggs and Egg Products. The most promising lines of endeavor seem to be (1) the use of the lipoids obtained by a chloroform-alcohol extraction, followed by the usual saponification; (2) solution of the lipoids obtained as in (1) in benzene, saponification with sodium ethylate, washing of the precipitated soaps with benzene, which dissolves sterols, and purification of the benzene solution by washing with water. The determination of sterols could be carried out on the unsaponifiable matter.

The associate referee followed the recommendation to continue the studies on the water-soluble protein-nitrogen precipitable by 40 per cent alcohol. The first conclusion drawn from this work was that there was no good reason for the title of this method. The constituent determined is practically all albumin. The title "crude albumin nitrogen" or "albumin nitrogen" is shorter and more significant than the present one, and should be substituted therefor.

As the result of an intensive study of the nitrogen method on alimentary pastes and on liquid and dried eggs, it was found necessary to go back to the indirect method, in which the nitrogen is determined before and after precipitation. This avoids the ever-difficult filtration and washing. It has been tried out by Palmer and the referee, and was subjected to collaborative work in 1928.[1] It is true that the results that year did not demonstrate any superiority for the indirect method, but more extended work by the referee this year disclosed a decided advantage in point of time and convenience, with no loss of accuracy. In the present direct method, it was found that occasionally a precipitate would become colloidal during the washing operation, and the determination would be lost.

Last year's report discussed the use of alumina cream as an aid to clearing and centrifugalizing and recommended more work with this substance, as well as with salt solution as solvent. It was found in this year's study that alumina cream carried down varying amounts of albumin, presumably by adsorption, for there appears to be no opportunity for mutual precipitation. Solutions of Merck's egg albumin shaken with alumina and iron creams, and allowed to stand overnight, settled completely, leaving clear solutions which gave no test for protein. The use of alumina cream was therefore discontinued. The use of salt solutions for extraction has also been dropped pending the results of some work on eggs.

[1] *This Journal*, 12, 398 (1929).

It is unfortunate that it has been found necessary to make some change in the nitrogen method almost every year. The referee considers it possible that another method, involving the use of a precipitant other than alcohol, may eventually be adopted. At another time it would be recommended that the present method remain unchanged until a sound,

TABLE 1.

Results for albumin nitrogen in flour and noodles.

SUBSTANCE	DIRECT METHOD (TENTATIVE)	INDIRECT METHOD (PROPOSED)
	per cent	*per cent*
Flour	0.028	0.026
	0.026	0.026
Flour	0.030	0.028
	0.027	0.028
Flour	0.017	0.028
	0.030	0.030
Water noodle	0.026	0.026
	0.023	0.027
Egg noodle	0.16	0.20
	0.19	0.20
Egg noodle	0.19	0.18
	0.19	0.19
Egg noodle	0.19	0.19
	0.21	0.19
Egg noodle	0.18	0.20
	0.11	0.18
Egg noodle	0.17	0.16
	0.09	0.16

reliable method suitable for all types of egg and cereal products had been developed and tested. But this year the imminent publication of the revised *Methods of Analysis* makes it desirable that no method which is certain to be changed in the near future be retained, and that whatever method is included should be the best available.

The method given below differs slightly from that studied in 1928, particularly in respect to the shaking. It has been found that vigorous mechanical agitation may cause coagulation of albumin; consequently, the directions provide for more gentle agitation.

ALBUMIN NITROGEN

Place 20 grams of the sample in an 8-ounce nursing bottle, add 100 cc. of water from a pipet, shake the bottle to prevent lumping of the sample, and add exactly

100 cc. more water. Mix the contents of the stoppered bottle gently by hand or on a slowly revolving wheel for 1 hour. (The temperature of the water should not exceed 30°C.). Centrifugalize to facilitate filtration and filter through a thin asbestos pad in a Hirsch funnel, using light suction. Determine nitrogen in 50 cc. of the filtrate as directed in the official method,[1] distilling the ammonia into 20 cc. of 0.1 *N* acid. Run a blank on the reagents. Pipet off 100 cc. of the above filtrate into a 200 cc. volumetric flask, add 15 cc. of sodium chloride solution (28 grams diluted to 300 cc.), fill nearly to the mark with 95 per cent alcohol, mix carefully to avoid foaming, cool to room temperature, make up to the mark with alcohol, mix well, and allow to stand overnight. Pipet off the supernatant liquid and filter through an 18½ cm. fluted filter paper. Determine nitrogen in 100 cc. of the filtrate as above. (In order to avoid bumping, it is advisable to add the sulfuric acid and boil off the alcohol before adding the potassium sulfate and mercuric oxide.) Subtract the value obtained from the water-soluble nitrogen to obtain the albumin nitrogen.

RECOMMENDATIONS[2]

It is recommended—

(1) That special study of methods for the determination of unsaponifiable matter in flour and in alimentary pastes be continued in conjunction with the same study on eggs.

(2) That the tentative method for the determination of water-soluble protein-nitrogen precipitable by 40 per cent alcohol be dropped.

(3) That the method for the determination of albumin nitrogen described in this report be adopted as tentative.

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(2) Analysis of Egg Pastes (macaroni). P. Leone, *Ann. chim. applicata,* **15,** 156 (1925).

(3) Cholesterol as a Measure of Egg Yolk in Milk Products. Lincoln M. Lampert, *Ind. Eng. Chem., Anal. Ed.,* **2,** 159 (1930).

(4) Report on Separation of Meat Proteins. W. S. Ritchie, *J. Assoc. Official Agr. Chem.,* **12,** 411 (1929).

REPORT ON COLLECTING AND PREPARING SAMPLE OF ALIMENTARY PASTE FOR ANALYSIS

By S. C. Rowe (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

The present tentative method[3] specifies that alimentary paste shall be quantitatively weighed and reweighed before and after grinding to pass a 20-mesh sieve when the total solids of the original unground material is desired. The associate referee prepared samples of macaroni in this manner and found that the loss in weight ranged from 0 per cent to 0.2 per cent,

[1] *Methods of Analysis, A.O A.C.*, 1925, 8.
[2] For report of Subcommittee C and action of the association, see *This Journal,* **14,** 66 (1931).
[3] *This Journal,* **9,** 43 (1926).

provided extreme care was exercised in the grinding and sifting operations. In the present method this loss in weight is calculated as moisture. It cannot correctly be assumed that the loss in weight is entirely moisture, since it is practically impossible to perform the grinding and sifting operation without a loss of material. By grinding no finer than a 20-mesh sieve, it is believed that little, if any, moisture loss occurs due to the heating of the mill. In any event, the loss in weight is slight regardless of how it occurs.

It is recommended that the tentative method for collecting and preparing a sample of alimentary paste for analysis be dropped, and it is suggested that in lieu thereof the following method be substituted:

Pick out sufficient strips from the lot to be analyzed to assure a representative sample. Break these into small pieces with the hands, mix, and grind 300–500 grams in a mill until all the material passes through a 20-mesh sieve. Keep in a sealed container to prevent changes in moisture.

REPORT ON MOISTURE IN ALIMENTARY PASTES AND BAKED PRODUCTS

By S. C. Rowe (U. S. Food and Drug Administration, Washington, D. C.), *Associate Referee*

In studying the present tentative method[2] for the determination of moisture in alimentary pastes and baked cereal products the associate referee, assisted by J. T. Keister of the Food and Drug Administration, prepared two different samples each of macaroni, bread, and cake. The moisture was determined by the air oven method for one hour at 130°C. and by the vacuum method for five hours at 98°C. with a pressure less than 25 mm. of mercury. The following results, expressed in percentage, were obtained:

	SAMPLE NO. 1		SAMPLE NO 2	
	AIR OVEN	VACUUM OVEN	AIR OVEN	VACUUM OVEN
Macaroni	10.20	10.08	10.18	10.08
Bread	10.66	10.59	10.83	10.75
Cake	9.05	8.70	10.30	10.06

It will be noted that the figures for macaroni and bread are reasonably close by the two methods, while the figures for cake by the air oven method are considerably higher than those obtained by the vacuum oven method. There was a noticeable browning of the samples of cake by the air oven method, which of course contained a much greater amount of carbohydrates and fat than the other products. Where the sample consists for the most part of cereal, as in the case of alimentary pastes and

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 67 (1931).
[2] *This Journal*, 9, 39 (1926).

bread, both methods appear to be equally applicable, but where the product contains large amounts of other substances, such as fats and carbohydrates, the air oven method appears to give too high results. From the results obtained in the past and those presented here it is recommended[1]—

(1) That the tentative method for the determination of moisture in alimentary pastes and bread be made official.

(2) That the tentative method for the determination of moisture in cake be further studied.

E. M. Bailey: I announced to you yesterday that we were scheduled to have the Secretary of Agriculture talk to us at this time. He is unable to be here, but we are fortunate to have a representative of the Department of Agriculture with us. Dr. Woods, who is Director of Scientific Work of the Department of Agriculture, has graciously consented to come over and talk to us for a while. I am glad to introduce Dr. Woods.

ADDRESS BY DR. A. F. WOODS, DIRECTOR OF SCIENTIFIC WORK, U. S. DEPARTMENT OF AGRICULTURE

The Secretary was extremely sorry that it was impossible for him to be here and meet you. He is out in the west working on some of these drouth problems and wasn't able to get back in time for this meeting, so he delegated me to come down here and say for him that he is very greatly interested in the work of this association. He knows a good deal about it. He knows that it is the basis on which a great deal of our regulatory work is established; the methods and procedures that are worked out by you research chemists and other investigators are the foundation work for procedures in all kinds of regulatory work, and he is interested in that and interested in the research that underlies it.

It seems to me that as I view the situation from the standpoint of these two organizations that are developing, the research group on the one hand, which I believe you people more particularly represent, and the regulatory group on the other, that some of the dangers which were foreseen in the segregation of these groups are beginning to appear and that it will be necessary to develop correlating machinery, correlating methods, means of closer contact between you people who investigate methods and the people who use these methods for the determination of facts upon which they base violations of law or in the Service groups upon which they base their recommendations for procedure. Now it is the easiest thing always for each man to keep on his side and attend to his own

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 67 (1931).

business, but in modern agriculture we are trying to get the team to pull together, trying to get all agencies that contribute to the solution of problems of an industry to work together with the view point of the service that the industry renders to the public. That means that the Research, Service, and Regulatory people must sit down around the table and each must learn something from the other. It isn't enough for those of us who are engaged in research to find facts and then think that we are through. It isn't enough for regulatory people to think that they can take some out-of-date material and use it as a means of enforcing the regulations when there is something better available. They need to make an effort to keep in touch with research people who should have the latest developments, and the research people need to keep in touch with the regulatory men. That requires a real effort, and a constant effort will have to be made to keep these groups working together. My job as Director of Scientific Work is to try to do that.

You people are noted for what you have accomplished in this direction of correlation. It has been one of the great services that you have rendered. I think many of our scientific organizations could well be guided by the history of this association. I have watched its growth for nearly 40 years, and while my work has never come very closely in touch with yours I have from time to time met a great number of your individual workers and have sat around the table with them and discussed these questions. I know your spirit and know it is right. I know that you need to assist some of the other groups to do the same thing.

Now research is every day finding new facts. We are constantly finding new facts in the field of vitamin research. Much work needs to be done by associations like yours in standardizing methods of work in determining vitamin potency. The presence of organic quantities of iodine, copper, and manganese in certain plant and animal tissues has a very definite relation to the public health. It is easy to neglect this, but it is a very important subject and one that official agricultural chemists and physiologists and men engaged in that type of work will need to study and to give careful consideration in relation to food values and the health of man and animals. We are doing a good deal of work in these fields in the Department of Agriculture and in experiment stations and other research agencies. For guidance in determining these factors, the Federal and State Service looks to you. I want to say for the Secretary, Chiefs of Bureaus, Directors, and the whole Department that we are always at all times ready to cooperate with you. If we seem to be going off by ourselves on some tangent, let us know about how it looks to you, as we want your suggestions and your criticisms.

After Dr. Wood's address Dr. C. A. Browne, Assistant Chief of the Bureau of Chemistry and Soils, related some of his experiences in foreign

countries, particularly in relation to modern developments of chemical procedure and methods. Dr. Browne's address was published on p. 101, Vol. 14.

No report on beers, wines and distilled liquors was given by the referee.

No report on specific gravity and alcohol was given by the referee.

REPORT ON VINEGAR

By A. M. HENRY (U. S. Food and Drug Administration, Philadelphia, Pa.), *Referee.*

In compliance with the recommendation of the referee for last year, methods for polarization on sulfates were studied. J. H. Fitelson, of the U. S. Food and Drug Administration, New York City, continued the study of a new glycerol method. No work on ash or phosphoric acid was undertaken.

A sample of cider vinegar, designated "A", and another sample of the same vinegar containing approximately one-half per cent of malic acid, designated "B," were submitted to collaborators, who were requested to make the following determinations:

(1) Polarize in a 200 mm. tube at 20°C. Report the result on the basis of a 200 mm. tube in degrees Ventzke.

(2) Decolorize 50 cc. of the sample with 1 gram of decolorizing carbon. Filter through a double filter and polarize in a 200 mm. tube at 20°C. Report the result on the basis of a 200 mm. tube in degrees Ventzke, stating the kind of carbon used.

(3) Decolorize 50 cc. of the sample by the addition of 10 cc. of alumina cream.[1] Filter through a double filter and polarize in a 200 mm. tube at 20°C. Correct reading for dilution and report the result on the basis of a 200 mm. tube in degrees Ventzke.

(4) Decolorize 50 cc. of the sample by the addition of 5 cc. of saturated neutral lead acetate solution; remove lead with powdered anhydrous potassium oxalate and again filter. Polarize in a 200 mm. tube at 20°C. Correct reading for dilution and report the results on the basis of a 200 mm. tube in degrees Ventzke.

A sample of the mixture of cider and molasses vinegar was submitted to collaborators who were requested to determine sulfates by the tentative method.[2]

The referee wishes to express his appreciation to the heads of the collaborating laboratories and to the following named chemists who did the analytical work:

1. Joseph P. Aumer, Food & Drug Administration, St. Louis, Mo.
2. Andrew G. Buell, Jr., Food & Drug Administration, Kansas City, Mo.
3. G. M. Bartlett, Agricultural Experiment Station, Orono, Me.

[1] *Methods of Analysis, A O.A.C.*, 1925, 182, 18(b).
[2] *Ibid.*, 329, 24.

TABLE 1.
Polarization results.

COLLABORATOR	SAMPLE A METHOD 1	2	3	4	VARIETY OF CARBON USED FOR METHOD	SAMPLE B METHOD 1	2	3	4	SAMPLE C SULFATES (SO_3)		
	°V.	°V.	°V.	°V.		°V.	°V.	°V	°V.			
(1)	−1.0	−0.8	−1.0	−1.9	Animal	−1.0	−1.0	−1.0	−1.1	11.1	−11.2	−10.9
(2)	−1.1	−0.8	−1.2	−1.1	Animal	−1.1	−1.3	−1.2	−1.1	10.9	−11.0	
(3)		—			—		—			11.2	−11.3	
(4)	−1.0	−1.0	−1.5	−1.1	Norit	−1.0	−1.0	−1.1	−1.7	10.3	−11.1	−10.6
(5)	−1.0	−0.6	−0.9	−1.1	Animal	−1.0	−0.7	−1.1	−1.2	10.3		
		−0.6			Purit		−0.6					
		−0.6			Darco		−0.6					
(6)	−0.6	−0.5	−0.7	−0.7	Animal	−0.9	−0.6	−0.8	−0.8	9.8	− 9.3	
(7)	−1.0	−0.7	−1.2	−1.1	Norit	−1.0	−0.8	−2.1	−1.2	11.0	−11.0	
(8)	−0.8	−0.9	−0.7	−1.0	Supra Norit	−1.0	−0.9	−0.8	−1.0	11.3	−11.1	
(9)	−0.6	−0.5	−0.7	−0.7	Norit	−0.7	−0.6	−0.6	−0.8	10.2	−10.3	
(10)	−0.9	−0.7	−0.9	−0.8	Not stated	−0.8	−0.6	−0.9	−1.2	9.0	− 9.1	− 9.7
(11)*	—	−1.0	−1.0	−1.1	Not stated	—	−1.0	−1.0	−1.1	10.0	− 9.8	− 9.8
(12)	−1.3	−1.0	−1.1	−1.1	Not stated	−1.2	−0.9	−1.1	−1.1	11.6	−11.5	
(13)		—			—		—			11.0	11.0	
(14)	−1.0	−0.7	−0.6	−0.8	Animal	−1.0	−0.7	−0.8	−0.9	9.1	−9.0	
Lowest	−1.3	−1.0	−1.5	−1.1		−1.2	−1.3	−1.2	−1.7	9.0		
Highest	−0.6	−0.5	−0.6	−0.7		−0.7	−0.6	−0.6	−0.8	11.6		
Average	−0.94	−0.72	−0.95	−0.95		−0.97	−0.79	−1.05	−1.10	10.50		

* Polarisation results not included in average.

4. Clyde H. Campbell, Consulting Chemist, Pittsburgh, Pa.
5. S. L. Crawford, Nat'l. Food Manufacturers Lab., Rochester, N. Y.
6. J. H. Fitelson, Food & Drug Administration, New York, N. Y.
7. M. J. Gnagy, Food & Drug Administration, Minneapolis, Minn.
8. J. F. Laudig, H. J. Heinz Co., Pittsburgh, Pa.
9. Paul A. Mills, Food & Drug Administration, Seattle, Washington.
10. J. I. Palmore, Food & Drug Administration, Washington, D. C.
11. Roy S. Pruitt, Food & Drug Administration, Cincinnati, Ohio.
12. L. A. Salinger, Food & Drug Administration, San Francisco, Calif.
13. Erwin Shupe, Food & Drug Administration, Chicago, Ill.
14. A. S. Wells, Oregon Dairy & Food Dept., Portland, Oregon.

COMMENTS BY COLLABORATORS

Polarization

Joseph P. Aumer.—A combination of Methods 2 and 3 has been found to give a solution which is practically decolorized and easily read in the polariscope. Twenty cc. of filtered solution No. 2 was shaken with 5 cc. of alumina cream and filtered. Animal charcoal was used for Method No. 2.

Clyde H. Campbell.—The samples treated with carbon had to be filtered several times before the readings were taken. The samples treated with alumina cream filtered rapidly and were clear but gave much higher results. Sample A was difficult to filter using lead acetate, but B filtered rapidly. It seemed that the samples treated with carbon gave more accurate readings than the untreated sample and were carefully handled without any necessary corrections, the chief objection being the double filtering.

S. L. Crawford.—(1) *Direct polarization* of samples submitted offers no difficulty. Vinegars much darker in color could not be read in a 200 mm. tube with usual light source.

(2) *Decolorizing carbon:* Boneblack—Harshaw Chemical Co., Cleveland, Ohio; Darco—Darco Sales Corp., New York, N.Y.; Purit—Baugh & Son, Philadelphia, Pa. All these materials give low results.

(3) *Alumina cream:* Gives polarization nearest to that of untreated sample. Filters readily and removes sufficient color to enable its use on vinegars much darker in color than samples submitted. Recommend further study as this clarifying reagent yields results closer to the true figures, particularly when an abnormal amount of malic acid has not been added.

(4) *Neutral lead acetate:* Sample A does not precipitate well with this reagent. Do not recommend the use of lead acetate for the reason that it removes certain dextrorotary substances, thus increasing the minus reading. There is also the disadvantage of an additional filtration as compared with alumina cream.

J. H. Fitelson.—The direct reading of the vinegars (Method 1), was not very sharp, particularly with the darker vinegar (A). Clarification efficiency was as follows: 1st, charcoal; 2nd, lead acetate; 3rd, alumina cream, alumina cream having about ½ the efficiency of the charcoal. Readings after use of charcoal show loss of optically active substances (Baker & Adamson's charcoal was used). Filtrates from lead acetate and alumina cream show no loss of optically active substances. It appears doubtful whether alumina cream by itself would decolorize dark colored vinegars sufficiently for accurate reading. The fixed acid in cider vinegars consists mostly of lactic acid, there being little or no malic acid in most cider vinegars. Therefore, the use of lead acetate appears to be safe except with those exceptional cider vinegars containing appreciable amounts of malic acid. The use of alumina cream is recommended wherever possible.

M. J. Gnagy.—Apparatus: A Ventzke polariscope, of ancient origin, put out by Julius Peters of Berlin, and imported by Arthur H. Thomas Co. of Philadelphia, was used. This polariscope is permanently set up in a corner of the laboratory where constant-temperature and light-control conditions are not available. The work was carried on during some of the hottest weather in August. All polarisation work was carried out in 200 mm. tubes.

Reagents: 1. *Decolorizing carbon:* "A" Norit (A48), put out by Eimer & Amend, was used. It has been in the laboratory over 2 years. 2. *Alumina cream:* The alumina cream was made up fresh before use and was prepared according to directions given. 3. *Neutral lead acetate solution:* A saturated solution of neutral lead acetate was made up a few hours before use by dissolving the C.P. chemical in cold, boiled, distilled water. 4. *Potassium oxalate:* The anhydrous C.P. salt was used to precipitate the excess of lead.

Methods: The methods followed were those given in the referee's letter of July 16, 1930, and the directions were followed literally. * * *

The portions of samples A and B under (1) and (4) of the table, which were first shaken with decolorizing carbon and alumina cream, were shaken at intervals for one hour except Sample A with carbon, which was shaken for a period of approximately 65 minutes. The portions of Samples A and B under (3) and (6) of the table were shaken with the carbon and alumina cream at intervals for only thirty minutes. The lengths of time taken for the precipitation with the lead acetate and for

TABLE 2.

Gnagy's corrected polariscope readings in degrees Ventzke taken with 200 mm. tubes at 20°C. on vinegar samples before clarification and after clarification by different reagents:

VINEGAR SAMPLES		ORIGINAL SAMPLE BEFORE CLARIFICATION	SAMPLE CLARIFIED WITH DECOLORIZED CARBON	SAMPLE CLARIFIED WITH ALUMINA CREAM	SAMPLE CLARIFIED WITH LEAD ACETATE AND POTASSIUM OXALATE
Sample A, (normal cider vinegar)	(1)	−0.96	−0.21 (a)	−1.16 (b)	−1.11 (d)
	(2)				−1.17 (f)
	(3)	—*	−0.71 (6) (c)	−1.21 (4) (c)	−0.95 (10) (f)
Sample B, (containing malic acid)	(4)	−0.94	−0.50 (b)	−2.33 (b)	−1.32 (a)
	(5)				−1.31 (f)
	(6)	−1.05 (10)	−0.76 (6) (c)	−2.07 (4) (c)	−1.16 (10) (f)

NOTES ON TABLE

Work on (1) and (4) was done July 30–Aug. 1; and on (2) and (5) on Aug. 5. Work on (3) and (6), except for original samples, was done Aug. 7–Aug. 9. Readings on the original samples before clarification, under (3) and (6), were made or attempted on Aug. 12.

* Sample too dark to read on Aug. 12. Read with difficulty on Aug. 1.
(a) Sample shaken at intervals with charcoal for 65 minutes.
(b) Samples shaken at intervals with clarifying reagent for 60 minutes.
(c) Samples shaken at intervals with clarifying reagent for 30 minutes.
(d) Four oxalate precipitates filtrations made before readings taken.
(e) Three oxalate precipitates filtrations made before readings taken.
(f) Two oxalate precipitates filtrations made before readings taken.

(10), (6), (4), represent number of readings made in pairs at intervals, from which averages taken for readings recorded. All other readings given represent average of ten consecutive readings.

the precipitation of the lead with the anhydrous potassium oxalate were variable, the filtration being carried out when the precipitation appeared to be complete. More than one filtration of the oxalate precipitate was made on the same solution.

The tubes containing the sample portions under (1), (2), (4) and (5) of the table were kept at constant temperature in a water bath cooled with ice when necessary. The sample portions under (3) and (6) of the table were cooled just below the temperature of the room and then transferred to the tubes which had been slightly cooled. The readings under (1), (2), (4) and (5) of the table are the average of ten readings made in succession by alternately turning in first from the left and then from the right. The other readings were made in pairs at intervals to avoid eye-fatigue, and the average of all readings made was then taken for the one given. The readings obtained under (3) of methods given in the letter were multiplied by the factor 60/50; those obtained under (4) by the factor 55/50. The results obtained are shown in Table 2:

COMMENTS

(1) Considerable difficulty was encountered in reading the polariscope. A spread of as much as 0.5 of a degree Ventzke was secured among ten readings on the same solution, depending on the nature of the solution. Erratic readings were sometimes secured on the same solution. Confirmatory readings were made at times by A. W. Hanson, C. B. Stone, and C. W. Harrison. Stone also made a series of readings on the same samples that the collaborator prepared and read under (1) and (4) of the table. In general his results were slightly lower except in the case of the solution clarified with decolorizing carbon when a larger variation in the opposite direction was noted. The spread of readings obtained by the same individual and the different readings obtained by different individuals are probably explained by eye-fatigue, by difference in sensitiveness of different persons' eyes to variation of color, and by the poor surroundings and conditions in which the polariscope was situated. The erratic readings were probably due to over-heating of the polarizing Nicol on the hot days on which the polariscope was read.

(2) The time allowed for shaking is apparently very important, especially in the case of the use of decolorizing carbon. There apparently is an adsorption effect of the carbon upon the substances which produce rotation. This adsorption increases with the time of contact. A variation in the intensity and frequency of shaking of the vinegar with the carbon will undoubtedly produce a variation in the results obtained. The nature of the carbon and its age will also have an important bearing upon its effectiveness.

(3) Sample B, the one containing considerable quantities of malic acid, shows an abnormal reading when clarified with alumina cream, a reading approximately twice the reading on the original sample. This might be explained on the assumption that the alumina cream has removed more of a substance with dextrorotary effect than it has of a substance with levoratory effect. One might further assume here that the time of contact of sample with the alumina cream had an effect as the reading obtained when the contact was an hour was higher than it was when the contact was thirty minutes. However, the difference is hardly sufficient to draw a definite conclusion on the data available.

(4) When Sample A was treated with 5 cc. of saturated lead acetate solution, a colloidal suspension remained above the precipitated material. On filtration, however, the filtrate passed through clear but very slowly. The lead acetate clarified Sample B readily.

(5) It was very difficult to remove the lead oxalate quickly and completely. It kept settling out from the solution after filtration. Two, three and even four filtrations were made. These extra filtrations apparently had no great effect upon the readings. No systematic effect was noticeable.

(6) Sample A, before clarification, was the more difficult one to read because it was darker in color. It was read with some difficulty on August 1. On August 12 another attempt at reading was made on a new portion taken from a small remainder in the bottle, but the vinegar had become so dark that a reading was impossible.

(7) Any effect on the reading that might have been due to variation in temperature was lost in the variable reading that was secured.

SUPPLEMENTAL REPORT BY GNAGY

Another portion of Sample B vinegar, which contained a considerable amount of malic acid, was used. The same polariscope was used under the same conditions as in August except that the temperature of the room was much lower. Four different batches of alumina cream were used. These are described as follows:

A.C. No. 1: This was the alumina cream used in the previous experimental work, except that it had received further washing to remove sulfate. This cream, on standing overnight, about half filled a 2.5 liter bottle. Daily washing of this alumina cream by decantation continued for a week or ten days after the July–August work was completed. It was allowed to stand until further work was requested. Then on attempting another washing by decantation the supernatant liquid became milky and colloidal. The qualitative test for sulfates in the supernatant liquid showed more sulfates present than for some time. This phenomenon increased for two more washings. Then two drops of strong ammonium hydroxide were added, and the contents of the bottle were shaken. The supernatant liquid then became clear, but great quantities of barium sulfate came down when the qualitative test was made on the decanted wash water. It took several more washings to remove the freed sulfates. As used finally this alumina cream showed less sulfate in the last washings than any of the other three creams. It was gelatinous in character.

A.C. No. 2: This alumina cream was precipitated on September 25th and washed rapidly by decantation. On standing overnight the precipitated aluminum hydroxide occupied only about one-third of the volume occupied by A.C. No. 1. The final washing before using showed by qualitative test the presence of slightly more sulfate than the final washing of A.C. No. 1 showed.

A.C. No. 3: This alumina cream is some old material, said to be about 4 years old, the authenticity of which is not vouched for. It was whiter, more granular, and denser, than the other cream, and of the consistency of milk of lime. A washing by decantation was made, and it showed by qualitative test slightly more sulfates than either A.C. No. 1 or A.C. No. 2.

A.C. No. 4: 60 grams of alum was dissolved in water to make a saturated solution. The aluminum was precipitated on September 30 and the liter beaker filled with water. Approximately 500 cc. was decanted off on each of four different washings. A great quantity of sulfates was present in the last wash water. It was desired that considerable sulfates be present in this alumina cream. This alumina cream was also gelatinous.

The method followed was used: Decolorize 50 cc. of the sample by the addition of 10 cc. of alumina cream.[1] Filter through a double filter and polarize in a 200 mm. tube at 20°C. Correct readings for dilution and report the result on the basis of 200 mm. in degrees Ventzke.

[1] *Methods of Analysis, A.O.A.C.*, 1925, 182, 18(b).

Eight different clarifications were made, two with each of the four alumina creams. In one set four 50 cc. portions of the vinegar were shaken with 10 cc. of each of the four creams for a period of 5 minutes. Ten shakes of the stoppered flask were made approximately every minute. The other set of four were shaken the same way at approximately the same intervals for one hour.

The clarified solutions were cooled just below the temperature of the room, poured into the 200 mm. tubes, also slightly cooled, and readings made immediately. All readings reported were made shortly after filtration was completed. The readings reported are averages of ten readings made on each solution. The readings obtained were multiplied by the factor 60/50 and the corrected readings reported.

The results obtained are shown in the following table:

Corrected polariscope readings in degrees Ventzke taken with 200 mm. tubes at 20°C. on a vinegar sample containing malic acid, after clarification with different alumina creams.

TIME OF CONTACT	A.C. NO. 1	A.C. NO. 2	A.C. NO. 3	A.C. NO. 4
5	−1.09 (1)	−0.98 (2)	−0.70 (3)	−2.11 (7)
60	−1.27 (4)	−2.10 (5)	−0.77 (6)	−2.35 (8)

(1) Work on (1), (2), and (3) was done on September 29; on (4), (5), and (6) on September 30; and on (7) and (8) on October 1.

(2) The filtration under (5) was very slow and the filtrate was not quite so clear as that under (2) although no paper could filter out anything further. The filtration under (7) was very slow. Both filtrations under (7) and (8) were completed about the same time, although the latter was commenced 55 minutes later. The filtrate under (8) appeared very clear to the eye, but with the polariscope it was the most difficult solution to read.

3. Analyst C. B. Stone made a series of check readings on all the above solutions, and in seven cases his results were slightly higher than those reported. His average reading under (6) was the same as the one reported.

Apparently the test for sulfates in the wash water from the alumina cream is not a sure indication of the amount of sulfates that may be held back by the precipitated aluminum hydroxide.

5. It would appear that the presence of varying amounts of sulfates has an effect on results obtained. In some way the sulfate apparently aids in removing a substance that affects rotation.

J. F. Laudig.—Method 1: Both samples A and B were too dark to be read directly in a 200 mm. tube. Readings were obtained with difficulty, a tube of 100 mm. length being used.

Method 2: Supra Norit was used as the decolorizing carbon. Difficulty was experienced in obtaining readings with the resulting filtrates due to the presence of colloidal carbon not removable by the finest filter paper available (Whatman 44). Clear filtrates, which were easily read with a 200 mm. tube, were obtained when an equal weight of a diatomaceous earth (Filter Cel) was mixed with the decolorizing carbon before addition to the vinegar. No difference could be detected in the readings of samples decolorized with and without the addition of the diatomaceous earth.

Methods 3 and 4: No difficulty was experienced with these methods. Clear filtrates were obtained and easily read in a 200 mm. tube.

J. I. Palmore.—All polarizations were made in a 200 mm. tube in a saccharimeter kept in a constant temperature room, except B (1) where a 100 mm. tube was employed. The result is expressed on a 200 mm. basis. A, (1), (2) and (3) were made at a temperature of 21.50°C.; A(4) was made at a temperature of 23°C.; B, (2), (3) and (4) were made at a temperature of 21.5°C.; and B (1) was made at a temperature

of 23°C. All polarisations in the above table represent the average of six readings except A (4) and B (1) where the average was obtained from twelve and nine readings, respectively.

R. S. Pruitt.—The vinegars decolorized with carbon were perfectly clear and readings were easily made. The vinegars decolorized with alumina cream were rather dark and the readings were somewhat harder to make. The vinegar "A" decolorized with neutral lead acetate formed a suspension which did not filter clear. When treated with a potassium oxalate, the solution filtered clear. The precipitate formed with lead acetate in vinegar "B" settled quickly. The readings could not be taken at the exact temperature of 20°C.

L. A. Salinger.—In respect to polarization determinations, trials were made on samples A and B, both filtered and unfiltered. In the 200 mm. tube solutions were so dark as to be unreadable. Readings were made in the 100 mm. tube on the filtered and unfiltered solutions. No appreciable differences were seen. Below, in table form, is a description of the appearance of solutions as prepared for reading:

Direct	*Decolorizing Carbon*	*Alumina Cream*	*Neutral Lead Acetate*
		Sample "A"	
Dark Hard to read	Water white. Very clear. Refiltered several times.	Dark, but clear. Refiltered (Sample "A")	Very pale yellow. Clear. Refiltered several times with lead. Very slow filtration. Very easy to read.
		Sample "B"	
Dark Hard to read	Water white. Very Clear. Refiltered several times.	Pale straw yellow. Clear. Refiltered.	Strawyellow. Clear. Very easy to read.

From the standpoint of easiness and consistency in the readings I should say that the solutions prepared with the neutral lead acetate were much more satisfactory than the other solutions. While not so white as the decolorizing carbon solutions, a small charge in rotation was more easily perceptible than in the other solutions. The other solutions appeared clear, but the field was not so sharp nor the change so definite as in the lead solution. More readings were taken than are reported. They were taken in groups. I selected the group that I thought was nearest to an average. The lead groups I believe contain the most satisfactory readings. On even such small readings a liberal allowance must be made. The readings on the lead were satisfactory, but on the other ones it seems almost like guesswork if anything more than approximation is desired. With our polariscope and the conditions of reading that prevail here, which I consider as good, possibly better than in most laboratories, I consider that there is an uncertainty of several tenths on vinegars, which of course makes a large per cent error. This uncertainty applies to the direct reading in a 100 mm. tube as well as the readings in the 200 mm. tube. This error would be cut somewhat under polariscopic and working conditions found in custom sugar work. This amount of uncertainty does not apply to the readings of vinegar which had been treated with the neutral lead acetate.

A. S. Wells.—I am rather favorable to the decolorization method with animal charcoal, although for years I have been under the impression that charcoal clarification was not satisfactory. I think the unfavorable report on charcoal clarification was on sugar solutions rather than on vinegars. Another point in favor of a charcoal decolorization method on vinegar is that there is no factor for dilution to figure as in methods three and four.

COMMENTS BY COLLABORATORS

Sulfates

Joseph P. Aumer.—No difficulty was experienced in making this determination.

Clyde H. Campbell.—Not having Monroe crucibles, I used special ashless paper which is prepared and hardened as SO_3. I usually use Gooch crucibles for this work and prefer them to the paper as it was necessary to filter the samples several times, which is not usually necessary with Gooch crucibles.

S. L. Crawford.—Barium sulfate precipitate weighed to 1 mg. Three determinations check. Ignited barium sulfate not pure white in color but brownish. Analysis of precipitate showed it to contain 0.35 mg. of Fe_2O_3. These results were corrected for iron oxide contained in precipitate. Sample of vinegar was found to contain: Iron (Fe) 0.0025 gram per 100 cc. The amount of iron present is approximately normal for a pure cider vinegar but is frequently found greatly in excess of this amount. Such being the case, an appreciable error may be introduced from precipitation of iron on boiling, and if so, suitable correction should be made, providing extreme accuracy is desired.

J. H. Fitelson.—The barium sulfate precipitate secured from vinegar C was dark brown in both cases. Long ignition did not influence the color of the precipitate. Analysis of this barium sulfate showed the presence of appreciable quantities of iron oxide. Tests for iron in all reagents used were negative. However, when the vinegar was acidified with HCl and tested for iron, both by the sulfocyanate and ferrocyanide tests, strongly positive reactions were secured. The sulfocyanate color was tested by shaking out with ether and then decolorizing with $HgCl_2$, both characteristic of ferric sulfocyanate. Various other vinegars were tested directly for ferric iron and in every case positive tests were secured, the strength of the tests increasing with the darkness of the vinegars. It is well known that the barium sulfate precipitated in the presence of ferric salts in the hot, will carry down some iron and may give results as much as 10% too low. (See Treadwell and Hall, Quantitative Analysis, 1928, p. 403.) Since Vinegar C was very dark colored and since the barium sulfate precipitate was contaminated with iron, the results are untrustworthy and probably too low. Iron contamination may explain the erratic results secured in previous A.O.A.C. collaborative work on vinegars. It is recommended that one of the modifications mentioned in Treadwell and Hall be tried, particularly precipitation of barium sulfate in the cold. The A.O.A.C. tentative method determines the SO_3 ion already present in the vinegar; ashing methods previously tried determine total sulfur as SO_3. Sulfur-dioxide-sugar combinations, present in sulfured dried fruits, are very stable and not easily oxidized, and it is quite possible that vinegar made from sulfured apple products may contain large amounts of unoxidized combined SO_2. It seems to me that this point needs clarification since the determination of total sulfur as SO_3 may be more valuable for detecting small amounts of evaporated apple products vinegar. The following experiment was carried out: Bromine water was made to 100 cc. of vinegar C until an excess was present and SO_3 was then determined in the usual manner; 11.2 mg. of SO_3 per 100 cc. was found. This increase is probably due to oxidation of organically combined sulfur to SO_3, but the possibility of determining total sulfur as SO_3 without the trouble of ashing is indicated here.

M. J. Gnagy.—The portion of the sample used was mixed with some ignited asbestos and filtered through a filter paper. The sulfates were precipitated about 4:30 P.M. one day and filtered off according to directions about 9:00 A.M. the following morning. The sulfates were filtered on ashless filter paper. Ashing was done in an electric furnace. The final ash was greenish-gray. Results were calculated from the 1930 atomic weights.

A question is raised regarding the filtering of the vinegar sample. On p. 325, Chapter XXIII, paragraph 2 reads: "Mix thoroughly and filter before proceeding with the analysis." The tentative method for sulfates on p. 329 reads "To 100 cc. of the sample that has been filtered absolutely clear through asbestos . . . etc." If the sample is filtered through a Gooch crucible with asbestos as an aspirator pump, loss of water and of volatile acids will undoubtedly take place and a consequent change in the constitution of the vinegar occur. If the vinegar is filtered through such a Gooch crucible with asbestos at atmospheric pressure, the asbestos mat may be washed loose and insoluble asbestos particles pass through with the vinegar, thereby increasing the weight of the barium precipitate. It is believed that one filtration of the vinegar through a good grade of filter paper without any asbestos should be sufficient for all analyses.

Minute traces of barium sulfate were discernible in the wash waters from the barium sulfate precipitates when they were allowed to stand.

No Monroe crucibles, or materials for making same, were available in the laboratory but judging from comment concerning same in the literature, one would believe they would be excellent for use in filtering off barium sulfate precipitates.

J. F. Laudig.—The barium sulfate obtained after ignition was white and apparently quite free from contamination.

J. I. Palmore.—In the determination of sulfates gravity filtration was used, and two ashless filter papers were used for each determination. The ash correction for these, almost negligible, was applied in all cases. A and B (2) were obtained by using Grade A Norit, a vegetable carbon. In the absence of positive knowledge as to what the true polarization of the vinegars should be, the analyst can only say that (2) is the easiest and (3) the most efficient clarifier from a standpoint of manipulation. If a number of determinations of sulfates are to be made, Monroe crucibles and muffle firing would work to a decided advantage.

L. A. Salinger.—In regard to the sulfates, blanks or controls were run. A correction of 0.0002 gram of $BaSO_4$ was obtained. Sample C was first filtered through paper. The barium sulfate was filtered on paper. All filtrations and washings were refiltered till clear and the precipitating beaker and funnel finally wiped out with filter paper. Ignitions were made in platinum crucibles by putting crucibles and contents in a cold muffle, heat being brought up slowly. Crucibles were held there until apparently all carbon was burned off (paper was smoked off), then ignited slightly over a free flame, weighed and reignited. No loss of weight occurred. The residue looked quite brown. The barium sulfate was then treated with one drop of concentrated HNO_3 plus one drop of concentrated H_2SO_4 and ignited cautiously to avoid spattering. Residue was now white with a slight reddish cast.

DISCUSSION

Polarization

Polarization was confined to the use of different methods where optically active non-volatile acid was present, which has been shown by Balcom and Yanovsky[1] to change the polarization materially when lead salts are used without being removed. The results of the collaborative work this year and of the writer's work last year have shown that decolorization with neutral lead acetate followed by removal of the excess lead has little effect on the polarization. The collaborative work indicates that a slight levorotary change occurs with the use of neutral lead acetate

[1] *This Journal*, 5, 245 (1921).

and the removal of excess lead when optically active non-volatile acid is present. The use of alumina cream also produces a slight levorotary change in polarization. Alumina cream does not remove the color so effectively as lead salts. In spite of the fact that Method 2 called for the use of vegetable carbon, five analysts used animal carbon, five other chemists used vegetable carbon, and three failed to state what kind of carbon was used. There seems to be no material difference in the effects of animal and vegetable carbon. The use of carbon as a decolorizing agent is very efficient, but gives a dextrorotary change in the polarization, whether optically active non-volatile acids were present or not.

Gnagy's results indicate that decolorizing carbon has a considerable effect on the polarization of vinegar, particularly if the action is increased by agitation and a longer time; consequently, he was asked to make a further investigation, and from his work it seems that the amount of sulfates in the alumina cream used has a decided effect on the polarization. Consequently, alumina cream is not considered suitable for a clarifying reagent.

The comments of the collaborators and the referee's experience would indicate that the use of normal lead acetate solution with removal is the most satisfactory reagent for clarification of vinegars for polarization.

Sulfates

Collaborators' results agree very well on the use of the present tentative method. These results confirm the results of the referee in 1927.[1] It is realized that this method does not show the total sulfur in the vinegar, but only that present as sulfates. Fitelson suggests the use of bromine water for oxidizing all the sulfur present to sulfate without ashing. This, and the work of the referee in 1927, where the vinegar was ashed, has shown only a small increase in sulfates and it is believed the present tentative method is as variable as these modifications. Therefore, the present method, with the elimination of the requirements for filtering through asbestos, is being recommended for adoption as official.

Glycerol

Fitelson continued the study of the glycerol method. He has proposed a new method which appears promising. It is now being tested by the writer.

RECOMMENDATIONS[2]

It is recommended—

(1) That Method 26, Alcohol Precipitate, tentative, and Method 27, Pentosans, official, be dropped.[3]

[1] *This Journal*, 10, 490 (1927).
[2] For report of Subcommittee C and action of the association, see *This Journal*, 14, 68 (1931).
[3] *Methods of Analysis, A.O.A.C.*, 1925, 330.

(2) That the following method for polarization be substituted for the present tentative method.

Whenever possible, polarize in a 200 mm. tube without decolorizing. Report results on basis of 200 mm. tube in degrees Ventzke. When necessary, decolorization may be accomplished as follows:

(a) To 50 cc. of the sample add a measured quantity of saturated neutral lead acetate solution, avoiding an excess of lead; filter; remove lead with powdered anhydrous potassium oxalate, and filter. Polarize and correct for the dilution with lead acetate solution.

(b) To 50 cc. of the sample add decolorizing carbon, avoiding an excess amount or length of treatment. Filter through a double paper and polarize.

(3) That Method 24, Sulfates, tentative, be amended by amending the first sentence to read as follows:

To 100 cc. of the absolutely clear sample add 2 cc. of approximately normal hydrochloric acid; heat to boiling; add 10 cc. of hot barium chloride solution (1 gram per 100 cc.), drop by drop; and continue the boiling 5 minutes, keeping the volume approximately constant by adding hot water from time to time as required.

(4) That methods for the determination of total and soluble ash be further studied with particular attention given to the use of sucrose or other substances for reducing the time of heating and to the temperature of ashing.

(5) That the methods for the determination of phosphoric acid be further studied in connection with studies on ash.

(6) That the method for the determination of glycerol be further studied.

(7) That the official method for the determination of total solids be studied, especially with reference to its application to vinegars high in solids, such as malt vinegars.

REPORT ON FLAVORS AND NON-ALCOHOLIC BEVERAGES

By JOHN B. WILSON (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

Following Recommendation 4 of the 1929 report, the referee has conducted a number of experiments with a view to modifying the proposed gravimetric method for the determination of total aldehydes in orange and lemon oils and/or extracts, but as yet no modification has been found which seems worthy of collaborative study. Several other methods have been found in the literature, which may be applicable to this determination, but the referee has not had an opportunity to investigate them sufficiently to decide whether or not they should be subjected to collaborative study.

RECOMMENDATIONS[1]

It is recommended—

(1) That the official Kleber method be removed from its place under the heading "Lemon and Orange Oils-Citral" and placed under the heading "Lemon and Orange Oils-Total Aldehydes" (final action).

(2) That more extensive collaborative work be done on the gravimetric method for the determination of total aldehydes in orange and lemon oils and/or extracts, described in last year's report of the referee or modifications of it, and that the search be continued for other methods that are applicable to both oils and extracts.

(3) That collaborative work be done upon the application of the tentative polariscopic method for the determination of oils of lemon, orange and limes in vegetable and mineral oils to solutions of these essential oils in glycerol and the acetic esters of glycerol.

REPORT ON MEATS AND MEAT PRODUCTS

By R. H. KERR (U. S. Bureau of Animal Industry, Washington, D. C.), *Referee*

The collaborative work consisted in a test of a method for determining moisture in meat by drying in air at a temperature well above 100°C. The method tested was developed by H. R. McMillin of the Washington Meat Inspection Laboratory of the Bureau of Animal Industry.

The method sent to the collaborators was as follows:

> Weigh accurately about 8–10 grams of the ground sample into a tared weighing bottle approximately 2 inches in diameter, containing a short glass rod flattened at one end, and spread out in a thin layer over the sides and bottom of the bottle by means of the glass rod. Dry in air at atmospheric pressure at a temperature of approximately 125°C. (not lower than 120° nor higher than 130°C.) for approximately 2-3 hours, or until no significant loss of weight occurs on subsequent drying for a period of 1 hour.

Three samples, one of fresh sausage, one of smoked and cooked sausage, and one of smoked and cooked sausage with cereal added, were sent to each collaborator.

A statement of the results is reported in the table.

The results are confirmatory of the conclusion reached by McMillin from his preliminary study, namely, that moisture can be determined in meat by drying in air at temperatures up to 130°C. as accurately as by drying in air at 100°C. Owing to the saving in time involved, it will be advisable in many cases to use the high temperature method. Accord-

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 68 (1931).

ANALYST	*Sample A—Fresh Sausage*				*Sample B—Smoked and Cooked Sausage*				*Sample C—Smoked and Cooked Sausage with Cereal Added*			
	MOISTURE BY DRYING IN AIR— AT 125°C.	AT 100–105°C.		PROTEIN ($N \times 6.25$)	MOISTURE BY DRYING IN AIR— AT 125°C.	AT 100–105°C.		PROTEIN ($N \times 6.25$)	MOISTURE BY DRYING IN AIR— AT 125°C.	AT 100–105°C.		PROTEIN ($N \times 6.25$)
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Wiley & Co. Baltimore, Md.	42.10	42.14		11.00	58.05	57.80		14.43	56.01	56.04		16.00
			In vacuum				*In vacuum*				*In vacuum*	
J. C. Blake	40.20		42.40	11.09	57.84		58.33	14.39	56.60		56.12	16.28
Inst. Am. Meat Packers	41.66		42.50		58.21		58.23		56.03		56.34	
Chicago, Ill.	43.45		42.46		57.99		57.96		56.10		55.94	
Frederick Fenger	42.54	42.15		11.25	58.45	57.95		14.75	56.22	56.17		16.30
Armour & Co.	42.44	42.23		11.62	58.25	57.87			56.37	55.92		16.60
Chicago, Ill.	42.15				58.27				56.27			
			By distilla-				*By distilla-*				*By distilla-*	
William Siebenberg	42.40		*tion with*	11.82	58.17		*tion with*	15.69	56.37		*tion with*	16.19
Schwarz Laboratories	42.30		*toluene*		58.14		*toluene*		56.57		*tolune*	
New York, N. Y.			42.31				57.91				56.34	
			In vacuum				*In vacuum*				*In vacuum*	
H. R. McMillin	42.19	41.91	42.11	10.94	57.92	57.72	57.91	14.07	56.37	55.90	56.15	15.93
Bur. Animal Industry Washington, D.C.	42.14	41.92	42.03	11.00	58.10	57.78	57.91	14.19	56.23	55.96	56.03	15.91
Wm. C. Owens	42.16	42.14		10.88	58.15	57.71		14.08	56.29	56.17		15.85
Bur. Animal Industry San Francisco, Calif.	42.55	42.21		10.90	58.01	57.96		14.03	56.21	56.15		15.72

F. B. Hilty	42.22	41.51	10.93	58.12	57.69		14.07	56.34	56.29		15.62
Bur. Animal Industry	41.95	41.71	10.79	57.90	57.79		13.84	56.37	56.19		15.69
Chicago, Ill.			10.83				13.85				15.71
			10.80				13.93				
David Edelman	42.13	42.14	11.00	58.32	57.83		14.00	56.67	56.12		15.82
Bur. Animal Industry	42 06	42 09	11.07	58 25	57.76		14.14	56.48	56.28		15.70
New York, N. Y.			11.00	57.82			14.19	56.21	56.21		15.82
W. B. Fromer	42.17	41.83		57.73	57.95			56.11	56.19		
Bur. Animal Industry	41.99	42.04		57.93	57 98			56.30	56.16		
St. Louis, Mo.											
Geo. L. McGinty	42.40	41.82	11.39	58.24	57.72		14.40	56.50	56.03		16.23
Bur. Animal Industry	42.26		11.31	58.18			14.33	56.20			16.04
St. Louis, Mo.											
I. M. Myers	41.68	41.85	10.95	57.99	58.03		14.03	56.43	55.95		15.85
Bur. Animal Industry											
Omaha, Nebr.											
W. C. McVey	42.25	42.20	10.84	58.30	58.10	57.95	14.23	56.38	56.06	55.89	16.24
Bur. Animal Industry	42.26	41.89	10.88	58.00	58.02	57.95	14.17	56.53	56.14	55.86	16.34
Kansas City, Kans.											

ingly, it is recommended[1] that the method studied be adopted as a tentative method.

In the 1925 edition of *Methods of Analysis*, pages 240 and 241, there appear two practically identical methods for the determination of nitrites in meat. One of these is sufficient. It is recommended, therefore, that the method designated as No. 14 be deleted and that designated as No. 14-A retained. The words, "according to the method for nitrites in water," in the next to the last line, should be changed to "as directed on page 85, No. 15."

The directions for the determination of amino nitrogen by the Sorenson Method, Chapter XVII, 34, are in some degree confusing. It is recommended that the first line of this method be striken out and the following substituted: "To 20 cc. of the filtrate from **27**, neutralized to phenolphthalein with barium or sodium hydroxide, or to 20 cc. of an equivalent. . . ."

It is also recommended that sections **37** and **38** of Chapter XVII, "Soluble Phosphorus in Blood, Brain, and Glandular Organs," be deleted, because these methods are no longer being used and are regarded as obsolete.

No report on the separation of meat proteins was given by the associate referee.

REPORT ON GELATIN

R. M. MEHURIN (U. S. Bureau of Animal Industry, Washington, D. C.), *Referee*

The work of the referee this year consisted of a study of the various methods for the determination of copper and zinc in gelatin, with a view to the selection of a method for the forthcoming revision of *Methods of Analysis*.

Previous reports of referees[2] on the results of collaborative work on the present tentative and alternative methods[3] have shown conclusively that these methods in the hands of the average chemist can not always be relied upon to yield accurate or uniform results. This is especially true in the determination of copper. Strong preference has been shown by referees and collaborators alike over a period of five years for the method based on the preliminary direct ashing of the gelatin in a muffled furnace[4] and more uniform and accurate results have been reported by referees by the use of such methods.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 57 (1931).
[2] *This Journal*, 8, 166 (1924); 9, 458 (1926); 12, 416 (1929); 13, 479 (1930).
[3] *Methods of Analysis*, *A.O.A.C.*, 1925, 256.
[4] *J. Ind. Eng. Chem.*, 15, 942 (1923).

After careful study, the referee has devised a method along these lines, which he believes will yield more reliable and uniform results, with less consumption of time than the present tentative methods. The method follows:

PROPOSED METHOD

Copper: Ash in a muffle furnace 50 grams of gelatin in a platinum or porcelain dish of 150–200 cc. capacity. Have the temperature of the muffle 500°–550°C., or a barely visible red, when the material is placed in it and maintain this temperature throughout the ashing. Cover dish with a watch-glass and dissolve the ash in 5–6 cc. of hydrochloric acid (1+1). If a carbonaceous residue remains, filter, wash with hot water, re-ignite, and treat as before. Evaporate the hydrochloric solution to dryness. Add 5–6 cc. of hydrochloric acid (1+1), filter into a 50 cc. Erlenmeyer flask, and wash with hot water to a volume of approximately 40 cc. Saturate the hot solution with hydrogen sulfide, stopper tightly, and allow to stand in a warm place for one-half hour or more. Filter into a 150 cc. Erlenmeyer flask and wash promptly and thoroughly with warm hydrochloric acid (1+20) saturated with hydrogen sulfide. Either dissolve copper sulfide on filter with hot nitric acid (1+1) or transfer the paper and precipitate to a small porcelain dish, ignite in a muffle furnace at a low temperature, cool, and moisten ash with 2 cc. of concentrated nitric acid. In either case evaporate the nitric acid solution to dryness on the steam bath. Dissolve the copper nitrate by heating on the steam bath, away from any H_2S fumes, for 5–10 minutes with 10 cc. of 10 per cent ammonium nitrate solution. Filter, if necessary, into a 50 cc. graduated flask, wash dish and filter paper, make up to mark, and mix. Measure out 10 cc. and 25 cc. into two 50 cc. Nessler tubes and to the 10 cc. portion add 3 cc. of 10 per cent ammonium nitrate solution. Make the tubes up to mark but do not mix. Prepare standard tubes containing 0.5–5.0 cc., in increments of 0.5 cc., of a copper solution containing 0.0001 gram of copper per cc. This solution is prepared by dissolving approximately 0.1 gram of pure copper in nitric acid, evaporating to dryness on the steam bath, boiling with 100 cc. of 50 per cent ammonium nitrate until dissolved, and diluting so as to contain 0.1000 gram of copper per liter. Add to each standard tube 5 cc. of 10 per cent ammonium nitrate solution and make up to mark, but do not mix. Add exactly 0.3 cc. of 1 per cent potassium ferrocyanide solution to both sample and standard tubes and mix. Match at once a sample tube with a standard tube.

Zinc: Boil the filtrate and washings from the hydrogen sulfide precipitation of copper until all the hydrogen sulfide is removed. Add 1 cc. of concentrated nitric acid and continue boiling to a volume of approximately 30 cc. Add strong ammonium hydroxide until definitely alkaline to litmus, heat to boiling, filter, and wash. If the precipitate is heavy, redissolve in hydrochloric acid, reprecipitate with ammonium hydroxide, and re-filter. Neutralize the filtrate to litmus with formic acid and add 1.0 cc. acid in excess, 2 cc. of 25 per cent sodium acetate solution and 1 cc. of 1 per cent mercuric chloride solution. Filter if not perfectly clear and free of sediment. Saturate the solution with hydrogen sulfide, stopper flask, allow to stand for 15 minutes, and filter through a quantitative paper. Wash thoroughly with warm filtered 1 per cent ammonium chloride solution saturated with hydrogen sulfide. Ignite in a tared platinum crucible at a dull red heat until completely ashed and then to a constant weight at a bright red heat. Weigh as ZnO. Weight of ZnO×0.8034 = weight of zinc.

The method for zinc is similar to that sent to collaborators last year for testing in comparison with the alternative method. One change is the sub-

stitution of 0.3 cc. of 1 per cent potassium ferrocyanide for 0.2 cc. of 4 per cent potassium ferrocyanide, the referee having found that the fading of the copper ferrocyanide, which heretofore has constituted the chief objection to the ferrocyanide determination of copper, was caused by the slight excess of the reagent that has been customarily used in the past. Another change made is the employment of 1 cc. excess of formic acid plus 0.5 gram of sodium acetate for the precipitation of zinc sulfide. This will furnish sufficient buffer of sodium formate and sufficient free formic acid to maintain a *p*H around 3, which is optimum for the precipitation of zinc sulfide and also will prevent the early precipitation of traces of iron usually present in ammonia and ammonium salts and likewise prevent the precipitation of traces of nickel sometimes found in gelatin. The extreme difficulty of filtering small amounts of zinc sulfide has led to the inclusion of mercuric sulfide as an entangling agent. Tests by the referee show that no loss of zinc sulfide is caused by the subsequent volatilization of the mercury. In this connection it should be noted that the mercury must be precipitated along with the zinc sulfide and in the presence of sufficient ammonium salts to prevent the formation of colloidal mercuric sulfide. For this reason ammonium chloride is included in the wash water. Phenolphthalein cannot be used as an indicator on account of the turbidity it causes in the solution prior to precipitation of the zinc sulfide. Litmus is adequate for the purpose.

One collaborator was secured, J. M. McCoy of the Bureau of Animal Industry. He had had no previous experience with the determination of copper or zinc in gelatin, nevertheless his results are excellent for copper and reasonably good for zinc. Known quantities of copper and zinc were added to gelatin.

	Copper		*Zinc*	
	ADDED *mg.*	FOUND *mg*	ADDED *p.p.m*	FOUND *p.p.m.*
	0.25	0.25	50	56
	0.35	0.30	50	69
J. M. McCoy	0.45	0.40	75	69
B. A. I.	0.55	0.50	75	83
	0.65	0.60	100	101
	0.75	0.60	100	104
	0.85	0.80	125	126
	0.95	0.80	125	137

Concerning the revision of the chapter on Gelatin in *Methods of Analysis*, it is recommended[1]—

(1) That the alternative method for the determination of copper and zinc in gelatin be deleted.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 69 (1931).

As stated above, collaborators have been unable to obtain consistent results with this method. While undoubtedly accurate in experienced hands for the determination of such amounts of copper as 2 mg. and upwards (40 p.p.m. and upward on a 50-gram sample) such comparatively large amounts of copper are now seldom found in gelatin.

(2) That the method for the determination of lead in gelatin be deleted.

No record of any test of this method could be found in the literature. In view of this fact and for the further reason that lead occurs but rarely in gelatin it is thought advisable to drop this method until such tests are made, or until a method is included in the chapter on Metals in Foods, to which reference can be made after suitable preparation of the sample for the determination of lead in gelatin.

(3) That the method for the determination of sulfur dioxide in gelatin be deleted.

No record of an adequate test of this method could be found. Mention is made, however, in the report of the referee for 1919, of determinations having been made in quadruplicate by Swift & Company, both by the distillation method and the diffusion method. The official distillation method gave consistent results in all four determinations as did also the diffusion method, but the results obtained by the latter method were only about 75 per cent of those obtained by the distillation method.

SELECTED REFERENCES

(1) U. S. Dept. Agr. Bur. Chem. Circ. **102**; *J. Soc. Chem. Ind.*, **26**, 1115 (1907).
(2) *J. Assoc. Official Agr. Chem.*, **14**, (1931).
(3) *J. Ind. Eng. Chem.*, **15**, 942 (1923).
(4) *J. Assoc. Official Agr. Chem.*, **5**, 343 (1922).
(5) *Ibid.*, **4**, 520 (1921).

REPORT ON SPICES AND OTHER CONDIMENTS

By KENNETH C. BEESON (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

The work this year consisted of a study of the various methods of determining lecithin phosphoric acid in mayonnaise. It is known that if a material is dried before extraction, the amount of lecithin phosphoric acid found is less than is actually present. Two methods that eliminate heating and drying are therefore recommended for future study: (1) the procedure outlined by Andrew,[1] and (2) the method of J. Grossfeld and P. Lederle.[2]

In some work done at the State Chemical Laboratory, Vermilion, S. Dakota, the referee found that 11 per cent more lecithin phosphoric acid

[1] *This Journal*, **8**, 700 (1925).
[2] *Z. Unters. Lebensm.*, **58**, 148 (1929).

was recovered by Andrews' method than by the present tentative method.[1] Any method proposed must be accompanied by a table of values of lecithin phosphoric acid in egg yolk determined in exactly the same way.

RECOMMENDATIONS

It is recommended—

(1) That the study of lecithin phosphoric acid determination in salad dressings be continued for the next year.

(2) That methods for the determination of starch and sugars in prepared mustard be studied next year.

(3) That the method proposed for the determination of reducing sugars before inversion be studied collaboratively along with the present tentative methods for total solids, oil, reducing sugars after inversion, and total acid in salad dressings.

REPORT ON CACAO PRODUCTS

By J. W. SALE (U. S. Food and Drug Administration, Washington, D. C.), *Referee*

In the associate referee's report on crude fiber in cacao products for 1929, a few results were given on crude fiber determined by both the tentative method for crude fiber[1] and the proposed method which is being recommended for adoption this year as an official method. Winkler of this Administration has extended this work of comparing the two methods on samples of both bitter liquors and sweet chocolate, with the results given in Table 1. The results for moisture and fat, and in the case of sweet chocolate, for sucrose also, are included in Table 1, as they are used to convert the figures for crude fiber, obtained by the tentative method, to a moisture-, fat-, and sugar-free basis. The results obtained by the two methods agree fairly well in view of their marked differences in procedure.

Some additional figures for factor F, referred to in the associate referee's reports on crude fiber for 1929–30, were obtained by Winkler. They are given in Table 2. The data in Table 2 agree very well with those reported by the associate referee.

In addition to the work described in the report on crude fiber for this year the associate referee conducted some work to develop a more direct and perhaps more accurate method for determining milk proteins in milk chocolate. The results obtained show the need of more work before a report is given. It is recommended, therefore, that the study of milk proteins in milk chocolate be continued next year. This work might well be

[1] *Methods of Analysis, A.O.A.C.*, 1925, 43, 322.

TABLE 1.

Crude fiber.

F. C. NOS. BITTER LIQUORS	PROPOSED METHOD[1]		TENTATIVE METHOD[2]		MOISTURE[3]		FAT[3]		SUCROSE[3]	
	Per cent		*Per cent*		*Per cent*		*Per cent*			
677-B	6.26		6.75	6.90	2.25		52.41	52.47		
678-B	6.57	6.43	5.56		2.22		52.15	52.11		
720-B	7.08	7.08	6.71	6.6		2.54	50.29	50.27		
682-B	6.5	6.0	7.53	7.54	1.96	1.96	54.5	54.5		
684-B	5.84	6.03	7.42	7.2	2.40	2.40	52.85	52.81		
686-B	6.88	6.94	7.7	7.68	2.22	2.22	51.4	51.63		
692-B	6.08	6.07	6.25	6.38	2.39	2.40	55.83	55.76		
694-B		5.77	5.42	5.62	2.85	2.84	53.02	53.10		
701-B	6.26	6.52	6.27	6.34	2.41	2.38	52.90			
706-B	6.20	6.24	6.18	6.6	2.13	2.17	54.0	54.09		
709-B	6.99	7.03	6.85	6.55	1.78	1.78	53.0			
711-B	6.9	7.0	7.07	7.15	1.93	1.98	53.74	53.76		
713-B	6.40	6.56	6.70	6.82	1.95	1.96	53.60	53.56		
716-B	6.82	7.17	7.24	7.59		1.74	50.65	50.56		
719-B	5.53	5.68	5.25	5.05	2.64	2.67		54.26		
Sweet Chocolate										
687-B	6.47	6.57	7.0	7.3	0.66	0.66	37.54	37.60	52.24	
696-B	5.91	6.1		6.32		1.56	38.29	38.31	32.06	32.16
707-B	6.61		6.8	6.9	0.60	0.60	34.6	34.8	56.46	56.39
714-B	6.46	6.51	6.5	6.63	1.27	1.29	33.52	33.52		47.83

[1] Referee's report 1929. Factor F = 29.85
[2] *Methods of Analysis, A O A C*, 1925, 343.
[3] *This Journal*, 9, 46 (1926).

TABLE 2.

Factor F in bitter chocolate liquors.

(percentage of water and alcohol-soluble material in fat-free material)

F. C. NOS.	FACTOR F[1]	
686-B	31.02	30.98
701-B	29.07	28.33
706-B	30.07	29.79
709-B	29.88	30.31
711-B	30.05	30.90
713-B	30.17	
716-B	28.54	29.14
Maximum	31.02	
Minimum	28.54	
Average	29.86	

[1] Determinations in duplicate.

combined with that on crude fiber in milk chocolate, continued work on which is being recommended by the Associate Referee on Crude Fiber.

The Associate Referee on Cacao Butter was unable to complete the work started on the Reichert-Meissl and Polenske numbers on cacao products containing coconut oil, palm-kernel oil, and butter fat in varying amounts. Some analyses reported indicate that when butter fat is present in a large amount of cacao butter, the butyric acid that is liberated from the soaps that are formed during the process passes over to a greater extent during the distillation of the fatty acids than that calculated from the Reichert-Meissl numbers of the butter fat and the cacao butter. Moreover, the Polenske number seems to be reduced. An illustration of this is as follows:

		REICHERT-MEISSL NO.	POLENSKE NO.
Butter		29.7	2.1
Cacao butter		0.27	0.20
Coconut oil		7.35	14.70
10% Butter fat 90% Cacao butter	observed	4.05	0.31
10% Butter fat 90% Cacao butter	calculated	3.21	0.39
90% Cacao butter	difference	+0.84	−0.08
10% Butterfat 10% Coconut oil 80% Cocao butter	observed	5.23	1.00
10% Butterfat 10% Coconut oil 80% Cacao butter	calculated	3.92	1.84
	difference	+1.31	−0.84

This work should be continued during the coming year as it is of importance from a regulatory standpoint.

During the year Fitelson of the New York Station, U. S. Food and Drug Administration, worked on a method for the determination of lactose and sucrose in milk chocolate by copper reduction. The removal of interfering non-sugar reducing substances is accomplished by precipitating them with an excess of mercuric nitrate in the presence of a slight excess of sodium bicarbonate. The results obtained indicate that the method is worthy of further study, and it is recommended that an associate referee be appointed to study the method.

Analysts frequently determine moisture in cacao products in an air oven at 100°C. instead of by the official method (in vacuo or in a current of dry hydrogen) as the amount of moisture in these products amounts only to a few per cent and it is believed that substantially the same results are obtained by the two methods. However, it was considered advisable to obtain some data on this point before recommending the air oven method as an alternative method. Winkler compared the two methods, and his results, contained in Table 3, show that either method gives equally good results.

TABLE 3.

Moisture in cacao products.

SAMPLE F. C. NO.	KIND OF CHOCOLATE	KIND OF DISH	AIR-OVEN METHOD	OFFICIAL METHOD	
			Per cent	*Per cent*	
711-B	Bitter	Platinum	1.93	1.93	1.98
694-B	Bitter	Platinum	2.78	2.84	2.85
692-B	Bitter	Aluminum	2.34	2.39	2.4
707-B	Sweet	Platinum	.52	0.60	0.60
714-B	Sweet	Platinum	1.32	1.27	1.29
687-B	Sweet	Aluminum	0.66	0.66	0.66
696-B	Sweet	Aluminum	1.66		1.56
698-B	Milk	Aluminum	.68	0.69	0.69
702-B	Milk	Platinum	1.06	1.06	1.06
717-B	Milk	Aluminum	1.92	1.67	1.64
697-B	Milk	Platinum	.79	0.80	0.81

The air oven method[1] is as follows:

MOISTURE.—TENTATIVE

Dry 2 grams of the sample, prepared as directed under 1, in a platinum dish in an air oven at 100°C. An aluminum dish may be used when ash is not determined on the same sample. Report the percentage loss in weight as moisture.

RECOMMENDATIONS[2]

It is recommended—

(1) That the following changes be made in the methods for the analysis of cacao products in the 1925 edition of *Methods of Analysis. Page 346*, **19**.—Determination.—Insert "(using 5 grams of fat)" at the end of the first sentence in the third paragraph. Delete the words "by more than 2 degrees" from the 6th line of the third paragraph. Insert in the 6th line of the third paragraph, between the words "butter" and "adulteration" the following sentence: "by more than 3 degrees in the case of fat from chocolate liquors or sweet chocolates, and by more than 6 degrees in the case of fat from milk chocolates." *Page 347*, **26**.—Change the heading "Reichert-Meissl Number.—Official." to "Reichert-Meissl and Polenske Values.—Official." Change "Proceed as directed on p. 290, **27**, or p. 291, **29**." to "Proceed as directed in ———."

(2) That the modified proposed method for the determination of crude fiber in bitter and sweet chocolate, described in the report on crude fiber for 1930, be adopted as official (first reading).

(3) That the method for the determination of moisture given in this report be adopted as a tentative method.

(4) That the study of a method for the determination of crude fiber in milk chocolate be continued.

[1] *Methods of Analysis, A. O. A. C.*, 1925, p. 343

[2] For report of Subcommittee C and action of the association, see *This Journal*, **14**, 69 (1931).

(5) That the determination of milk proteins in milk chocolate be studied.

(6) That the study of the determination of foreign fats in cacao butter be continued.

(7) That the chemical method for the determination of lactose and sucrose in milk chocolate, in which interfering non-sugar reducing substances are removed with excess of mercuric nitrate in presence of a slight excess of sodium bicarbonate, be studied.

REPORT ON CRUDE FIBER IN CACAO PRODUCTS

By MARIE L. OFFUTT (U. S. Food and Drug Administration, New York, N. Y.), *Associate Referee*

The referee's report last year[1] gave a modification of the method for crude fiber proposed previously for use on bitter and sweet chocolates. A factor (F = % water and alcohol-soluble material in the ether-dried residue) was also proposed to change the arbitrary figure (D) for crude fiber by the proposed method to the formal standard one (E).

Table 1 of this report gives results for F, D and E on 12 additional samples. It is interesting to note that the results on Nos. 69, 16, 825 and 824, which are alkalized products, do not vary from the results obtained on other numbers which are not alkalized. The average F for 9 samples last year was 29.7 per cent, and for 12 samples this year it was 29.98 per cent. The average of the combined 21 samples, 29.85 per cent, was used in the collaborative work this year.

TABLE 1.

*Results for F, D, and E.**

SAMPLE	F per cent	D per cent	E per cent
824	28.45	9.44	6.75
825	30.48	9.34	6.49
69	30.81	9.03	6.25
16	28.35	9.10	6.52
773	29.04	9.09	6.45
132	30.94	8.95	6.18
069	30.51	9.30	6.46
064	30.05	8.27	5.78
044	30.57	10.25	7.12
048	30.24	10.09	7.04
803	29.91	9.85	6.90
806	30.41	9.22	6.42

* Average F for 12 samples here —29.98 %
Average F for 9 samples last year—29.7 %
Average for 21 samples —29.85 %.

[1] *This Journal*, 13, 482 (1930)

Two samples, a bitter chocolate and a sweet chocolate made from the bitter, were sent out to collaborators with the following instructions:

CRUDE FIBER

(Results to be expressed in percentage. Duplicate determinations should be run if sample permits. Report both D and E.)

On *Bitter Liquor* and *Sweet Chocolate* determine the crude fiber by the following method:

Treat 7 grams of liquor or 50 grams of sweet chocolate with 100 cc. of ether in a nursing bottle, centrifugalize, and decant the supernatant liquor twice; dry the residue in an oven at about 100°C. and powder the residue in the bottle with a flattened glass rod. In some cases it may be found necessary to grind the material in a mortar and extract a third time with ether. Wash in the nursing bottle with three 100 cc. portions of distilled water at room temperature, shaking well each time until no cocoa material adheres to the bottle. Centrifugalize after each washing for 10–15 minutes and decant the aqueous layer. Wash the residue in the same fashion with two 100 cc. portions of 95% alcohol and one 100 cc. portion of ethyl ether. Transfer the residue to a platinum dish, dry to constant weight at 100°C, and grind in a mortar. Weigh 2 grams of the dried material and determine crude fiber by the usual A.O.A.C. method. Note: Linen should be used for both acid and alkaline filtrations in all the crude fiber determinations. Convert the crude fiber (D) obtained to standard one (E) by use of the factor F = 29.85% by the following equation:

$E = D - (D \times F)$, in which
D = % crude fiber in washed and dried material
F = % water-, acid-, and alcohol-soluble material in ether-dried residue
E = % crude fiber on moisture-, fat-, and sugar-free basis.

It was later decided to simplify this equation to $E = 0.7D$[1] for future use.

The chemists reporting and to whom acknowledgment is made are:

(1) H. A. Reed, Food and Drug Administration, Seattle, Wash.
(2) W. C. Taber, Food and Drug Administration, San Francisco, Cal.
(3) M. L. Offutt, Food and Drug Administration, Chicago, Ill.
(4) Thomas C. Dunn, Food and Drug Administration, Chicago, Ill.
(5) W. T. Mathis, Connecticut Agricultural Expt. Station, New Haven, Conn.
(6) M. M. Jackson, Food and Drug Administration, Buffalo, N. Y.
(7) W. O. Winkler, Food and Drug Administration, Washington, D. C.
(8) D. W. McLaren, Food and Drug Administration, Philadelphia, Pa.

The results of the collaborators are summarized in Table 2. The results of six of the eight varied less than 1 per cent for D on both the bitter and sweet chocolate, and the average D for bitter and sweet chocolate varied less than 0.1 per cent.

The associate referee made three determinations for F on the bitter liquor used and found 28.76, 28.86 and 28.68 per cent, respectively, thus giving an average of 28.77 per cent as against the average F = 29.85 per cent used, which would make about 0.1 per cent difference in E when calculated from D.

[1] Results in Table 2 were calculated from F = 29.85%.

TABLE 2.

Crude fiber.

COLLABORATOR	BITTER CHOCOLATE				SWEET CHOCOLATE			
	D		E		D		E	
	per cent		*per cent*		*per cent*		*per cent*	
(1)	9.33		6.54		9.55		6.70	
	9.51	Av. 9.42	6.67	Av. 6.61	9.73	Av. 9.64	6.83	Av. 6.77
(2)	8.56		6.01		9.18		6.44	
	8.66	Av. 8.61	6.08	Av. 6.06	9.32	Av. 9.25	6.54	Av. 6.49
(3)	9.54		6.69		9.16		6.43	
	9.39	Av. 9.47	6.59	Av. 6.64	9.56	Av. 9.36	6.71	Av. 6.57
(4)	8.56		6.02		7.60		5.36	
(5)	9.42		6.61		9.51		6.67	
	9.47	Av. 9.45	6.64	Av. 6.63	9.83	Av. 9.67	6.90	Av. 6.79
(6)	9.78		6.86		10.14		7.12	
	9.42	Av. 9.60	6.61	Av. 6.74	10.01	Av. 10.08	7.02	Av. 7.07
(7)	9.65		6.77		9.85		6.91	
	9.57	Av. 9.61	6.71	Av. 6.74	9.87	Av. 9.86	6.92	Av. 6.92
(8)	7.33		5.14		6.70		4.70	
	7.15	Av. 7.26	5.02	Av. 5.09	6.93	Av. 6.89	4.86	Av. 4.84
	7.30		5.12		7.05		4.95	
Maximum	9.61		6.74		10.08		7.07	
Minimum	7.26		5.09		6.89		4.84	
Average	9.00		6.32		9.04		6.34	
Variation	2.35		1.65		3.19		2.23	

The crude fiber in milk chocolates was studied further, but the results indicated that more work must be done before a method is proposed.

RECOMMENDATIONS

It is recommended—

(1) That the modified proposed method for the determination of crude fiber in bitter and sweet chocolates be adopted as official (first reading).

(2) That the study of a method for the determination of crude fiber in milk chocolates be continued.

No report on cacao butter was given by the associate referee. See report of Referee on Cacao Products.

REPORT ON COFFEE

By P. A. CLIFFORD (Food Control Laboratory, U. S. Food and Drug Administration, Washington, D. C.), *Referee*

The association has done no work on coffee since 1921, and the last report was made in October of that year.

During this time the problem of the determination of caffeine in so-called "decaffeinated" coffees has arisen. Several investigators, notably Allen,[1] have shown that the methods in which the percentage of caffeine is based upon the weight of the final residue give erroneous results in the case of coffee low in caffeine content. Working with the official Power-Chesnut and the tentative Fendler-Stüber methods, Allen has shown that in the case of decaffeinated coffees the residue from the chloroform extractions is only from 20 to 40 per cent caffeine; and while the experimental error arising from the weight of non-caffeine residue is not excessive in the case of ordinary coffees, in the case of coffees low in caffeine content it gives results which are wholly erroneous.

However, caffeine can be determined accurately from the nitrogen content of the residue, and in fact the Fendler-Stüber method requires, and the Power-Chesnut method recommends, this refinement. Allen[1] and Röttinger[2] have estimated caffeine in decaffeinated coffees by a microdetermination of nitrogen in the residues with satisfactory results.

A series of analyses was made with a market brand of decaffeinated coffee to see if the existing methods would give satisfactory results; and if not, to determine what refinements were necessary. The caffeine extractions were made by the official Power-Chesnut method, because it is considered theoretically more desirable than the Fendler-Stüber method. Lepper[3] has shown that the potassium permanganate solution used in the Fendler-Stüber method destroys traces of caffeine.

Nitrogen was determined in the residues from the chloroform extraction by several methods, and the results were compared. Also an effort was made to determine the caffeine in the residues by sublimation.[4] The comparative results are given in Table 1.

It is seen at once that percentages based directly upon the weight of residue are much too high. This is to be expected as the residues were always contaminated with brown waxy impurities obviously not caffeine. The average of 14 determinations by the Power-Chesnut method was 0.067 per cent.

Four determinations of nitrogen on the residues obtained by the Power-Chesnut method were made by a microchemical method. Pregl

[1] *This Journal*, **13**, 265 (1930)
[2] Microchemie, Pregl-Festschrift, 1929, pp. 308–312.
[3] *This Journal*, **4**, 526 (1921)
[4] *Ibid.*, **13**, 267 (1930); Oehrli, H A , Promotions arbeit-Tech. Hochschule, Zürich. Druck von Thomas & Hubert, 1923.

TABLE 1.

Power-Chestnut method

(Percentage of caffeine)

(Nitrogen Determinations By Micro-Kjeldahl Method)

DETERMINATION NO.	BY WEIGHT OF RESIDUE	BY NITROGEN DETERMINATION	SUBLIMATION	
			BY WEIGHT	BY NITROGEN DETERMINATION
1	0.061	0.0217		
2	0.056	0.0210		
3	0.062	0.0207		
4	0.065	0.0204		
		Av. −0.0209		
5	0.088		0.029	0.0182
6	0.053		0.039	0.0183
7	0.075		0.025	0.0189
8	0.073		0.019	0.0132
			Av. of 3 .031	0.0185
(Nitrogen Determinations by Ordinary Kjeldahl Method.)* (Duplicates combined in order to furnish a larger sample—*N*/10 Solutions used).				
9	0.062	0.0259		
10	0.063			
11	0.067			
12	0.067	0.0259		
		Av. −0.0259		
(Nitrogen Determinations with Micro-digestion and Ordinary Kjeldahl Apparatus For Distillation. *N*/50 Solution).*				
13	0.072	0.0253		
14	0.068	0.0232		
Av.	−0.067	Av. −0.0242		

* These determinations were made with the assistance of Mr. Benenson of the Nitrogen Laboratory of the Bureau of Chemistry and Soils.

tubes were used for digestion, and a modified Parnas-Wagner apparatus was used for distillation. The average of these four determinations, expressed as caffeine, was 0.0209 per cent. (For purpose of comparison the result is carried out to the fourth decimal.) The digestion mixture consisted of 40 mg. of mercuric oxide, 1.5 cc. of sulfuric acid and 0.75 gram of potassium sulfate and *N*/50 solutions were used in the titrations. The referee places great confidence in this micro method because repeated tests showed it to be accurate to at least 0.02 mg. For instance, the average of five determinations gave a recovery of 2.01 mg. from samples containing 2.0 mg. of pure caffeine.

These results were compared with those obtained by the ordinary Kjeldahl method. In this case the residues from two duplicate determinations were combined in order to furnish a larger sample and reduce the experimental error; *N*/10 solutions were used in the titrations. The average of 2 determinations was 0.0259 per cent, reported as caffeine, a result that compares quite favorably with that obtained by the micro method.

Two further determinations were made by means of the ordinary Kjeldahl method, slightly modified. In this case the crude caffeine residue was digested in the Pregl tubes as in the micro method. The mixture was then washed into ordinary 500 cc. Kjeldahl flasks, and the distillation was carried out in the usual way with $N/50$ solutions and with the further precaution of thoroughly steaming out the distillation apparatus immediately before distillation, as recommended by Allen. The average of two results by this procedure was 0.0242 per cent, which also checks well with the figures obtained by the two other methods. This latter figure approximates more nearly the result by the micro method, which is thought to be most nearly correct.

An effort was made to purify the residues by sublimation and to compare these results with those obtained by the nitrogen determinations. For this purpose a Hortvet sublimator[1] was used.

Considerable trouble was encountered in the attempt to secure a pure sublimate, and it was necessary to carry out the sublimation under carefully controlled conditions in order to obtain a sublimate of even approximate purity. In fact the best determination gave a sublimate only 77 per cent pure in caffeine. The contaminator was a brown oil which was apparently nearly as volatile as the caffeine itself. At first a temperature of 150–160 was utilized, but when it was seen that the sublimed caffeine was not pure, a lower temperature was used. Best results were obtained with a temperature of 90–100° for a period of 10–12 hours. Sublimation is complete in this time as was shown by raising the temperature to 250° for 2 hours. This higher temperature yielded a trace of a waxy impurity but no additional caffeine. Determinations No. 5 and 7 were made with a temperature of 100° for 10–12 hours. Determination No. 8 was made at 80–90° for 7½ hours, but sublimation was incomplete. (These results were not included in the average.) In all cases pressure was lowered to 5–7 mm. of mercury by means of a vacuum pump.

There is a certain amount of non-caffeine nitrogen left in the residue after sublimation of the caffeine is complete. This non-caffeine nitrogen amounted to 11 per cent of the total nitrogen in No. 7.

It is seen that caffeine values by nitrogen on the sublimate give very constant results seemingly in spite of the weight of the sublimate. This led the referee to believe that the average figure of 0.0185 per cent is very near the truth. Caffeine percentage by nitrogen direct on the residues (micro method) is 0.0209, but this includes the non-caffeine nitrogen which the sublimation apparently eliminates. However, the discrepancy is not great, and it is felt that caffeine can be estimated with sufficient accuracy by means of a nitrogen determination on the impure residue.

[1] *This Journal*, 6, 481 (1923).

CONCLUSIONS

(1) In the case of de-caffeinated coffees values for caffeine based upon the direct weights of the extracted residue are erroneous.

(2) A nitrogen determination permits the estimation of the amount of caffeine present with sufficient accuracy.

(3) This determination may be made with micro methods, or, if suitable precautions are taken, by means of ordinary methods.

RECOMMENDATIONS[1]

It is recommended that further work be done on this problem for the purpose of perfecting methods and with a view towards collaborative study.

The referee wishes to acknowledge his thanks to E. P. Clark of the Bureau of Chemistry and Soils for valuable advice regarding the micro Kjeldahl procedure.

REPORT ON NAVAL STORES

By F. P. VEITCH (Bureau of Chemistry and Soils, Washington, D. C.), *Referee*

There has appeared no need to modify the existing methods of the association for examination of rosin or turpentine oil, and no cooperative work has been done within the association on either subject during the past year.

RECOMMENDATIONS[2]

In order that the methods of this association may be in strict harmony with those of the American Society for Testing Materials and of the Federal Specifications Board, it is recommended that the present method for specific gravity (tentative) be deleted and the following methods for specific gravity (tentative) be adopted.

Determine the specific gravity at 15.5/15.5°C. by any convenient method that is accurate within 2 points in the fourth place. If the determination is made at any other temperature, which is not advisable, correct the reading by adding or subtracting 0.00082 for each degree Centigrade that the temperature at which the determination is made is respectively above or below 15.5°C.

It is also recommended that work on naval stores be continued with special reference to methods for the analysis of rosin.

No report on turpentine was given by the associate referee.

No report on paints, paint materials, and varnishes was given by the referee.

[1] For report of Subcommittee C and action of the association, see *This Journal*, 14, 57 (1931).
[2] For report of Subcommittee B and action of the association, see *This Journal*, 14, 50 (1931).

CONTRIBUTED PAPERS

DETECTION OF ADDED LECITHIN IN CHOCOLATE PRODUCTS

By W. O. WINKLER, with J. W. SALE (Food Control Laboratory, Food and Drug Administration, U. S. Department of Agriculture, Washington, D. C.)

OBJECT OF THE INVESTIGATION

Manufacturers and distributors of soya bean lecithin and technical advisers to the chocolate and confectionery industries have been advocating the use of this substance in the manufacture of chocolate coatings. It is claimed that from 0.2 to 0.3 per cent of added lecithin, equivalent to about 0.6 to 0.9 per cent based on the fat content, will increase the fluidity of chocolate coatings, thus saving a substantial amount of cocoa butter, and will retard graying or "budding" of chocolate confectionery.[1] It has also been asserted by some manufacturers that the normal content of lecithin in cacao beans is reduced in the process of manufacture.

The purpose of this investigation was to ascertain whether or not the presence of added lecithin could be detected by objective examination, and whether or not lecithin is actually lost in the process of manufacture, as claimed.

LECITHIN VS. ORGANIC PHOSPHORUS

In this investigation the organic phosphorus extracted by ether, petroleum ether, alcohol, and absolute alcohol, separately or in combination, is used as an index of the lecithin content. Whether or not the phosphorus obtained in this way truly represents the actual lecithin, the difference in organic phosphorus extracted from a cacao product, if determined by the same procedure before and after the addition of lecithin, should serve as a measure of the amount of lecithin added even though the actual lecithin cannot be calculated from the organic phosphorus with strict accuracy. The percentage of lecithin reported herein was obtained by multiplying the organic phosphorus found by 26.

DATA OBTAINED BY OTHER ANALYSTS

A general discussion of the selective solubility and effectiveness of various solvents in extracting lipoids from plant tissues is contained in the article, "Lipides and Their Estimation in Vegetable Tissues" by Charles E. Sando[2] and will not be repeated here. This article was prepared at the request of the Committee on Methods of Chemical Analysis for the American Society of Plant Physiologists. It contains an extensive bibliography.

[1] U. S Patent, 1,781,672
[2] *Plant Phys.*, 3, 155–184 (1928).

Data on lecithin content of cacao products reported by other analysts are meager. H. Jäckle[1] found 0.0073 per cent phosphorus corresponding to 0.189 per cent of lecithin (factor 25.9) in a single sample of cocoa butter. Fincke,[2] thinking that the phosphorus content of cocoa butter might enable the analyst to identify cocoa butter manufactured by the use of solvents, obtained the following results:

	Phosphorus *per cent*	Lecithin* *per cent*
Cocoa butter by expression	0.0039	0.101
Cocoa butter by expression	0.0036	0.094
Cocoa butter by expression from alkalized cocoa	0.0014	0.036
Cocoa butter by expression from unalkalized cocoa	0.0015	0.039
Cocoa butter—extracted	0.0010	0.026
Cocoa butter—extracted	Trace	
Cocoa butter—extracted	?	
Cocoa butter from commercial cream chocolate	Trace†	

* Calculated by the authors by use of factor 26.
† Extracted with ether.

Fincke made a few analyses of a blend of Accra, Thomé, and Java beans to show the effect of roasting on the phosphorus content of cacao butter. He used ether for a solvent and obtained the following results.

	Phosphorus (based on fat) *per cent*	Lecithin* (based on fat) *per cent*
Unroasted beans	0.0024	0.062
Moderately roasted beans	.0039	.101
Strongly roasted beans	.0074	.192

* Calculated by the authors by use of factor 26.

Since the apparent increase in phosphorus content on roasting was considered remarkable by Fincke, he made some further tests to show the effect of varying the quantity of solvent used and the variety of bean and obtained the following results:

	Ether used per 100 grams cacao mass *cc.*	Phosphorus (based on fat) *per cent*	Lecithin* (based on fat) *per cent*
Accra beans (Forastero)	250	0.0039	0.101
Accra beans (Forastero)	750	.0038	.099
Java beans (Criollo)	250	.0111	.289
Java beans (Criollo)	750	.0114	.296

* Calculated by the authors by use of factor 26.

The following data on the lecithin content of cacao beans and sweet chocolate coating were obtained by a commercial chemist in the latter part of 1930 and reported to the senior author in a letter.

	Lecithin (based on fat) *per cent*
Fancy Venezuelan cacao bean	0.49*
Dark sweet chocolate coating	.16†
Dark sweet chocolate coating	.09†
Dark sweet chocolate coating	.15†

* The solvents, alcohol-ether, were removed at low temperature, and lecithin was precipitated with chilled acetone. It was not clear from the report whether the lecithin was reported on the bean or fat basis.
† Solvent was petroleum ether.

[1] *Z. Unters. Lebensm.*, 5, 1062–77 (1902).
[2] Die Kakaobutter und ihre Verfalschungen, 1929.

The following data were reported by Dr. Bruno Rewald in an article entitled "Die Bedeutung des Lecithins für die Schokoladen-Industrie."[1]

Variety of bean	Phosphatide in dried mass of cacao beans *per cent*
Accra	0.127
Arriba	.256
Bahia	.071
Porto Cabello	.068
Thomé-Cabello	.118
Superior Sommer Arriba	.176
Superior Bahia	.102

Phosphatide (lecithin) in cacao products	*per cent*
Ordinary cocoa	0.52
Cacao mass	.33
Lecithin-chocolate	.36
Full milk chocolate	.24
Choc Choc Suchard	.094
Melted (Schmelz) Chocolate	.24

No analytical methods or other information regarding the above data are contained in the article.

METHODS USED IN THIS INVESTIGATION

Method I

The solvent used was absolute alcohol. The sample was extracted 10 hours.

This method is essentially the same as that designated "Lecithin-Phosphoric Acid (P_2O_5)—Tentative" in "*Methods of Analysis A. O. A. C.*, 1925," 322. The modifications made necessary by the character of the sample used follow:

Mix 5–10 grams of the chocolate sample with about 10 grams of clean sand and several grams of powdered $CaCO_3$ and transfer the mixture to a continuous extractor of the siphon type.

With commercial lecithin, the procedure from this point was exactly the same as that given in the tentative method. Further modifications used with the samples of chocolate liquor follow:

After 10 hours' extraction with absolute alcohol, decant the extract and distil off the solvent under reduced pressure at a temperature below 50°C. Take up the fatty residue in $CHCl_3$ and filter through cotton in a funnel. Wash the residue in the funnel several times with $CHCl_3$ and transfer the portion remaining in the beaker to the funnel with the aid of a glass rod. Proceed as in the tentative method, beginning "add 5 cc. of alcoholic potassium hydroxide solution." Multiply the quantity of phosphorus found by the factor 26 and report as lecithin.

Method II

The solvent used was absolute alcohol, and the extraction was con-

[1] Bulletin Official International Des Fabricants De Chocolat et de Cacao, March, 1931.

TABLE 1. *Comparison of methods for determination of lecithin.*

METHOD	SOLVENT USED	KIND OF SAMPLE	LECITHIN FOUND (PER CENT)	
			BASED ON SAMPLE	BASED ON FAT
I	Absolute Alcohol—10 hrs.	Commercial lecithin in vehicle of soybean oil No. 1	27.1	
	" "	" " " "	21.8	
	" "	" " " "	28.6	
III(a)	Ethyl Ether—Alcohol	" " " "	58.5	
	" " "	" " " "	59.1	
V(a)	Pet. Ether—Alcohol	" " " "	59.8	
II	Absolute Alcohol—4 Exts.	Commercial sample lecithin and cocoa butter No. 2	37.4	
III(a)	Ethyl Ether—Alcohol	" " " "	52.7	
IV(a)	Petroleum Ether	" " " "	54.0	
V(a)	Petroleum Ether—Alcohol	" " " "	54.2	
V(b)	Pet. Ether—Absolute Alcohol	" " " "	53.7	
III(a)	Ethyl Ether—Alcohol	Commercial Sample Milk Powder	0.16	
V(a)	Pet. Ether—Alcohol	" " " "	0.23	
I	Absolute Alcohol—10 hrs.	Authentic Bahia Liquor 684B	0.31	0.59
II	Absolute Alcohol—4 Exts.	" " " "	0.30	0.57
V(a)	Petroleum Ether—Alcohol	" " " "	0.38	0.72
V(b)	Pet. Ether—Abs. Alcohol	" " " "	0.31	0.59
I	Absolute Alcohol—10 hrs	Authentic Accra Liquor 692B	0.33	0.59
II	Absolute Alcohol—4 Exts.	" " " "	0.12*	0.22
III(a)	Ethyl Ether—Alcohol	" " " "	0.23	0.41
III(a)	" " "	" " " "	0.25	0.45
III(a)	" " "	" " " "	0.24	0.43
III(a)	" " "	" " " "	0 26	0.45
IV(a)	Petroleum Ether	" " " "	0.15	0.27
IV(a)	" "	" " " "	0.19	0.34
V(a)	Petroleum Ether-Alcohol	" " " "	0.28	0.50
V(b)	Pet. Ether—Absolute Alcohol	" " " "	0.26	0.46
VI	Pet. Ether—Pption with acetone	" " " "	0.10	0.18
VI	" " " " "	" " " "	0.12	0.22
VI	" " " " "	" " " "	0.11	0.20
VI	" " " " "	" " " "	0.12	0.22
I	Absolute Alcohol—10 hrs.	Authentic Arriba Liquor 709B	0.32	0.60
II	Absolute Alcohol—4 Exts.	" " " "	0.05*	0.09
V(a)	Petroleum Ether—Alcohol	" " " "	0.25	0.47
V(b)	Pet. Ether—Absolute Alcohol	" " " "	0.22	0.42
I	Absolute Alcohol—10 hrs.	Authentic Liquor (50 Accra—50 Bahia 719B	0.39	0.72
IV(b)	Petroleum Ether	" " " " "	0.16	0.29
V(a)	Petroleum Ether Alcohol	" " " " "	0.40	0.74
III(a)	Ethyl Ether—Alcohol	Authentic Liquor 719B with 1% Com. Lecithin No. 1	0.84	1.52
IV(b)	Petroleum Ether	" " " " "	0.51	0.92
IV(b)	" "	" " " " "	0.43	0.78
V(a)	Petroleum Ether—Alcohol	" " " " "	0.93	1.68
I	Absolute Alcohol—10 hrs.	Stock Mixture (Liquor 692B) with 5% Com. Lecithin No. 3	2.8	4.60
III(a)	Ethyl Ether—Alcohol	" " " "	2.8	4.6
III(a)	" " "	" " " "	2.8	4.6
V(a)	Petroleum Ether—Alcohol	" " " "	3.1	5.1
V(a)	" " "	" " " "	3.0	4.93

* Solvent cooled, causing fat to separate.

TABLE 2.

*Completeness of extraction of lecithin by using method V(a) (Petroleum Ether-Alcohol).**

LAB. No-B	KIND OF SAMPLE	LECITHIN FOUND (PER CENT)			
		METHOD V(a)		EXTRACTION REPEATED	
		BASED ON SAMPLE	BASED ON FAT	BASED ON SAMPLE	BASED ON FAT
672	Factory roasted and cleaned Arriba nibs	0.33	0.64	0.02	0.04
673	Factory roasted and cleaned Bahia nibs	.31	.63	.04	.08
676	Factory roasted and cleaned Trinidad nibs	.32	.64	.06	.12
681	Factory roasted and cleaned Accra nibs	.30	.55	.04	.08
877	Unroasted hand-shelled Accra beans	.32	.58	.04	.07
"	" " " " "	.22	.40	.03	.05
"	Roasted 100°C. " " "	.24	.38	.02	.04
"	" 110°C. " " "	.20	.36	.06	.11
"	" 110°C. " " "	.23	.42	.05	.09
"	" 130°C. " " "	.32	.58	.05	.09
"	" 140°C. " " "	.34	.61	.03	.05

* Samples were reground in mortar before re-extractio

TABLE 3.

Recovery of lecithin added to liquors by method III(a) (Ethyl Ether-Alcohol) and V(a) (Petroleum Ether-Alcohol).

METHOD	SOLVENT USED	KIND OF SAMPLE	LECITHIN PRESENT PER CENT		LECITHIN FOUND PER CENT	
			BASED ON SAMPLE	BASED ON FAT	BASED ON SAMPLE	BASED ON FAT
III(a)	Ethyl Ether-alcohol	Authentic Accra Liquor 692-B with 0.4% commercial lecithin, No. 3	0.47	0.84	0.35	0.62
"	" "	" " " "	0.47	0.84	0.37	0.66
"	" "	Authentic Accra Liquor 692-B with 1.2% commercial lecithin, No. 3	0.93	1.63	0.68	1.19
"	" "	" " " "	0.93	1.63	0.63	1.10
"	" "	Authentic Accra Liquor 692-B with 5.0% commercial lecithin, No. 3	3.2	5.26	2.8	4.6
"	" "	" " " "	3.2	5.26	2.8	4.6
V(a)	Petroleum Ether-Alcohol	Authentic Accra Liquor 692-B with 1.0% commercial lecithin, No. 3	0.86	1.51	0.88	1.55
"	" "	" " " "	0.86	1.51	0.84	1.48
"	" "	Authentic Accra Liquor 692-B with 5.0% commercial lecithin, No. 3	3.2	5.26	3.1	5.1
"	" "	" " " "	3.2	5.26	3.0	4.9
I	Absolute Alcohol—10 hrs.	" " " "	3.2	5.26	2.8	4.6

ducted at a temperature below 50°C. (This procedure was developed because E. B. Working stated that the solvent should be removed at a temperature below 50°C.)

Place 15 grams of chocolate liquor in a 250 cc. centrifuge bottle. With commercial lecithin samples, take about one gram and mix with several grams of powdered $CaCO_3$.

Extract with warm absolute alcohol (45–50°C.) by shaking with a rotary motion for about 2½ minutes, then place the sample in a water bath at a temperature of 46–50°C. for 15 minutes. Remove from bath and shake 2½ minutes, replace in bath for 15 minutes, again shake for 2 minutes, and centrifugalize for 10 minutes. Remove the bottle from the centrifuge and place in a water bath for 3 or 4 minutes at 45–50°C. Transfer the sample to a distillation flask by decantation and wash out any adhering fat from the neck and mouth of the bottle with alcohol at 50°C.

Repeat the extraction described above four times. Place the combined extracts in a distillation flask and remove the solvent under reduced pressure at a temperature below 50°C. Follow the procedure outlined in Method I, beginning "Take up the fatty residue in chloroform."

Method III (a) Ethyl ether—alcohol

The solvent used was a mixture of ethyl ether and alcohol. This method is essentially the tentative method used for the determination of lipoids and lipoid phosphoric acid (P_2O_5) in liquid eggs, as described in *This Journal*, 9, 58 (1926). The necessary modifications made for the samples used in this investigation follow:

Place 15–20 grams of cacao product (or 0.5–1.0 gram of sample mixed with several grams of calcium carbonate in the case of commercial lecithin) in a 250 cc. centrifuge bottle and proceed as in the tentative method just referred to, except to use 30–35 cc. of alcohol for digestion in place of 15 cc. and 60–70 cc. of ether after cooling. When the $CHCl_3$ solution has been filtered, proceed as in Method I, beginning "Add 5 cc. of alcoholic potassium hydroxide solution." Conduct the shaking operation at all times by a rotary motion.

Method III (b)

The solvent used was a mixture of ethyl ether and alcohol and the method used was the following:

Follow the same procedure outlined in Method III (a) to the completion of the filtration of the $CHCl_3$ solution. Saponify this solution with alcoholic KOH, evaporate to dryness, and dissolve the soaps in water. Acidify with nitric acid (1+3), let stand, and filter off the fatty acids. Determine phosphorus in the acid aqueous liquor after evaporating to dryness with HNO_3, as directed in *Methods of Analysis A.O.A.C.* 1925, 3, 7 or 10.

Method IV (a)

The solvent used was petroleum ether.

Extract 15 or 20 grams of chocolate liquor (or 0.5–1.0 gram of commercial lecithin) with 100 cc. of petroleum ether in a 250 cc. centrifuge bottle, shaking 4 or 5 minutes with a rotary motion. Centrifugalize and decant the supernatant liquid. Repeat the

extraction with two 50 cc. portions of solvent and evaporate the combined extracts to dryness. Take up the fatty residue in $CHCl_3$, filter, and proceed as directed in Method I after filtering the $CHCl_3$ solution.

Method IV (b)

Follow the procedure outlined in Method IV (a) to the filtration of the $CHCl_3$ solution. Saponify this solution and follow method III (b) to completion.

TABLE 4.

Lecithin found in cacao beans and chocolate liquors by Method V(a) *(petroleum ether—alcohol).*

LAB. NO (B)	KIND OF BEANS	LECITHIN FOUND PER CENT	
		BASED ON SAMPLE	BASED ON FAT
	Unroasted hand-shelled beans		
876	Haiti	0.45	0.83
878	Costa Rica	.28	.51
879	Java	.46	.88
880	Ecuador	.37	.74
875	African	.43	.80
877	Accra	.26*	.47
881	Trinidad	.40	.78
	Factory roasted and cleaned nibs		
676	Trinidad	0.32	0.64
710	Trinidad	.39	.72
672	Arriba	.33	.64
708	Arriba	.30	.57
673	Bahia	.31	
683	Bahia	.36	.68
693	Bahia	.35	.66
681	Accra	.30	.55
671	Accra	.36	.69
705	Accra	.30	.56
691	Accra	.36	.64
685	Sanchez	.34	.66
	Chocolate liquors—Factory ground—Authentic		
684	Bahia	0.38	0.72
694	Bahia	.33	.62
686	Sanchez	.37	.72
709	Arriba	.25	.47
711	Trinidad	.31	.58
706	Accra	.29	.54
682	Accra	.33	.60
692	Accra	.28	.50

* Average of 0.32, 0.26, 0.24 and 0.22%.

Method V (a)

The solvent used was a mixture of petroleum ether and alcohol.

Place 15–20 grams of sample (or 0.5–1.0 gram of commercial lecithin) in a 250 cc. centrifuge bottle and extract with 100 cc. of petroleum ether, shaking with a rotary motion for several minutes. Centrifugalize and decant the supernatant liquor and wash any adhering fatty material from the mouth and neck of the bottle with petroleum ether, using a wash bottle. Make the alcohol digestion as in Method III (a), cool, and add twice as much petroleum ether as the alcohol taken. Follow Method III (a) from this point on, substituting petroleum ether in all cases for ethyl ether.

TABLE 5.

*Effect on lecithin content of roasting hand-shelled Accra and Trinidad beans.**

LAB. NO. (B)	METHOD	SOLVENT USED	KIND OF BEANS	TIME AND TEMP. OF ROASTING	LECITHIN FOUND PER CENT			
					BEFORE ROASTING		AFTER ROASTING	
					BASED ON SAMPLE	BASED ON FAT	BASED ON SAMPLE	BASED ON FAT
				hours° C.				
877	V(a)	Pet. Ether-Alcohol	Accra	½ 100	0.26†	0.47	0.21	0.38
"		" " "	"	" 110	0.26	0.47	0.20	0.36
"		" " "	"	" 110	0.26	0.47	0.23	0 42
"		" " "	"	" 130	0.26	0.47	0.32	0.58
"		" " "	"	" 140	0.26	0.47	0.31	0.56
881	I	Absolute Alcohol—10 hrs	Trinidad	" 110	0.44	0.85	0.40	0.78
"		" " "	"	" 140	0.44	0.85	0.44	0.85
"		" " "	"	" 180	0.44	0.85	0.39	0.76
"	II	Absolute Alcohol—4 Exts	"	" 110	0.43	0.83	0.39	0.76
"		" " "	"	" 140	0.43	0.83	0.36	0.70
"		" " "	"	" 180	0.43	0.83	0.36	0.70
"	Va	Pet. Ether-Alcohol	"	" 110	0.42	0.81	0.43	0.83
"		" " "	"	" 140	0.42	0.81	0.43	0.83
"		" " "	"	" 180	0.42	0.81	0.42	0.81

* The cacao beans were roasted in a laboratory size coffee roaster.
† Average of 0.32, 0.26, 0.24 and 0 22%

Method V (b)

The solvent used was a mixture of petroleum ether and absolute alcohol.

Proceed as directed in Method V (a), except to make the alcohol digestion with absolute alcohol in place of 95 per cent alcohol.

Method VI

The solvent used was petroleum ether. The lecithin was precipitated with acetone and alcoholic $MgCl_2$ solution. This method is a modification of one used by W. R. Bloor for the determination of lecithin in blood serum.[1]

Proceed as directed in Method IV (a) to the filtration of the $CHCl_3$ solution. Precipitate the lecithin from the $CHCl_3$ solution in the centrifuge bottle by adding

[1] *J. Biol. Chem.*, 82, 273 (1929).

three volumes of chilled acetone and three drops of a saturated solution of $MgCl_2$ in alcohol for each 2 cc. of $CHCl_3$ solution. Centrifugalize and decant the excess liquid. Wash the sample several times with chilled acetone, centrifugalize, and decant. Dissolve the precipitated lecithin in ethyl ether saturated with water, saponify, and determine phosphorus as outlined in Method I, or oxidize by wet combustion and determine phosphorus according to *Methods of Analysis*, *A.O.A.C.*, 1925, 3, 7 or 10.

EXPERIMENTAL

The results obtained by the use of the procedures described above are given in Tables 1–6 inclusive.

TABLE 6.

Comparison of content of lecithin in shell and in hand-shelled unroasted beans by method V(a) (*petroleum ether—alcohol*).

LAB NO. (B)	KIND OF BEANS	LECITHIN FOUND PER CENT		
			SHELLED BEANS	
		SHELL BASED ON SAMPLE	BASED ON SAMPLE	BASED ON FAT
878	Costa Rica	0.13	0.28	0.51
877	Accra	0.10	0.32	0.58
881	Trinidad	0.12	0.40	0.78

DISCUSSION OF DATA IN TABLES

Methods I and II (absolute alcohol) and V (a) and (b) (petroleum ether with alcohol or absolute alcohol) gave results on the liquors which were higher than those obtained by the other methods and which agree fairly well with each other (Table 1). It is believed that these methods are capable of giving the most accurate results because by their use the lecithin is apparently more thoroughly extracted. Of these four methods, the two which involve the use of alcohol or absolute alcohol as the sole solvent are more time-consuming than Methods V(a) and (b), in which petroleum ether-alcohol is used. Absolute alcohol gave abnormally low results on the samples of commercial lecithin (Table 1), apparently owing to the physical condition of the samples. Even though these samples were mixed with sand to increase contact with the solvent, undissolved sample was noticeable after the extraction. Methods III (a) and (b) (ethyl ether—alcohol) gave results which were somewhat lower than those obtained by the use of alcohol or petroleum ether—alcohol (Table 1). Methods IV (a) and (b) (petroleum ether) gave substantially lower results on the liquors than did the other methods (Table 1). These methods, therefore, are not suitable for the determination of lecithin in chocolate liquors but appear to be unobjectionable for the determination of lecithin in cocoa butter. There is little choice between Methods V (a) and V (b), the former giving slightly higher results than the latter (Table 1). Also,

alcohol which is used in the former method is less expensive than absolute alcohol. Therefore, of all the methods which were tested, V (a) (petroleum ether—alcohol) is considered to be the most desirable and is the one recommended by the authors for the determination of lecithin in cacao products. This method was used in carrying out most of the latter part of this investigation.

Method VI, in which the fat was extracted with petroleum ether and the lecithin precipitated with acetone and $MgCl_2$, gave only about one-third the amount of lecithin found by Methods I and V (a) and (b) on sample 692-B (Table 1). These low results were no doubt due in large part to the use of petroleum ether as a solvent, but the precipitation with acetone and $MgCl_2$ apparently had some effect also in producing the low results.

The percentage recovery of lecithin by Method V (a) (petroleum ether —alcohol) varied from 77 to 94, with an average recovery of 87 per cent (Table 2). These samples were ground by hand and were reground before repeating the extractions. It is probable, therefore, that the recovery on commercial samples of chocolate would be much higher than is indicated by the data in this table.

The lecithin found agreed with the calculated amount when Method V (a), employing petroleum ether-alcohol, was used (Table 3). The other methods did not give such good results.

The lecithin content varied from 0.47 to 0.88 per cent based on fat, in the seven varieties of unroasted, hand-shelled beans tested and from 0.55 to 0.72 per cent based on fat, in the twelve samples of different varieties of factory roasted and cleaned nibs examined (Table 4). This variation is somewhat less than that shown with the unroasted hand-shelled beans probably because five instead of seven varieties of beans were represented. The lecithin content of factory liquors from different varieties of beans varied from 0.47 to 0.72 per cent on a fat basis (Table 4).

The data in Table 5 show definitely that the roasting has but little if any effect on the lecithin content. The slight loss shown is by the absolute alcohol method, which is not believed to be quite so reliable as the petroleum ether-alcohol method. The representations which have been made to the effect that the process of manufacture, chiefly the roasting, reduces the lecithin content to 0.05 to 0.01 per cent are shown by these data to be unfounded.

The lecithin in the three samples of cacao shell examined (Table 4) varied from 0.10 to 0.13 per cent based on weight of sample taken. Since cacao shell contains about 8.5 per cent of fat[1] it is apparent that the lecithin in shell on a fat basis is several times that in shelled cacao beans expressed on a fat basis.

[1] U. S. Dept. Agriculture Bull. 1413.

CONCLUSIONS

(1) A method for the determination of lecithin in cacao products, based on the use of petroleum ether and alcohol, is described, and data are given to establish its superiority over five other methods.

(2) The lecithin content of 27 samples of raw and roasted beans and liquors, representing 10 varieties of beans, varied from 0.47 per cent to 0.88 per cent based on fat. The authors have been advised by chocolate manufacturers and distributors of lecithin that the proper amount of lecithin to add to sweet coatings is about 0.3 per cent, which is equivalent to about 1 per cent on a fat basis. The above data show that by employing the method of analysis recommended herein, the addition of lecithin to coatings in the ordinary commercial quantities can be readily detected and the amount of added lecithin calculated with a fair degree of accuracy.

(3) Contrary to representations made by certain chocolate manufacturers, no material change in the lecithin content was noted when cacao beans were roasted at temperatures and for periods of time corresponding to commercial practice.

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COLORIMETRIC METHODS FOR THE DETERMINATION OF MANGANESE IN PLANT MATERIALS*

By JEHIEL DAVIDSON and RUTH G. CAPEN (Food Research Division, Bureau of Chemistry and Soils, U. S. Department of Agriculture).

The development of convenient analytical apparatus and easy and accurate methods of analysis is always followed by an increased volume of useful research.

* Food Research Division Contribution No. 126.

Thus the potassium periodate method adopted by the Association of Official Agricultural Chemists for plant analysis has stimulated research relating to the role of manganese in plant and animal life and to the manganese content of food stuffs. However, other methods are available for the colorimetric determination of small quantities of manganese. The lead peroxide method suggested by Crum[1] in 1845 was the first attempt to determine manganese colorimetrically. Because this method has been universally considered to be unsatisfactory, it has not been used in this investigation. The sodium bismuthate method, however, suggested by Reddrop and Ramage[2] in 1895, and the ammonium persulfate method, suggested by Marshall[3] in 1901, were widely used for the estimation of small quantities of manganese until the discovery of the potassium periodate method by Willard and Greathouse.[4]

The adoption of the periodate method by the A.O.A.C. was not preceded by extensive collaborative work, and there is no record of any collaborative work to test the comparative merits of the principal colorimetric methods used by different investigators. The object of this investigation, therefore, was to compare the periodate method with the sodium bismuthate and the ammonium persulfate methods and also with the gravimetric method for determining manganese in plants outlined in the official methods.[5]

MATERIALS AND PROCEDURES

Five cereals, wheat straw, and four leafy vegetables with a sufficiently wide range of variation in their manganese content were selected for analysis. Two inorganic manganese compounds, potassium permanganate and manganic oxide (Kahlbaum's), were also analyzed.

The periodate method used was a modification reported previously by the writers.[6] Phosphoric acid was used in preparation of the solutions. This modified procedure overcomes without special precautions the difficulties encountered by Richards.[7] Skinner and Peterson[8] also found that this modified procedure is helpful in overcoming the interference of calcium salts in analyzing animal materials for manganese. The sodium bismuthate method used was the modification outlined by Gortner and Rost,[9] who used a high concentration of sulfuric acid to hasten the oxidation of the manganese and to prevent the precipitation of basic bismuth salts. The ammonium persulfate method as outlined by Newcomb and Sankaran[10] was used.

[1] *Ann. Chem. Phar.*, 55, 219 (1845).
[2] *J. Chem. Soc.*, 67, 775 (1895).
[3] *Chem. News*, 83, 76 (1901).
[4] *J. Am. Chem. Soc.*, 39, 2366 (1917). Complete bibliography on development of colorimetric methods for the estimation of manganese till 1916.
[5] *Methods of Analysis, A.O.A.C.*, 1925, 40.
[6] *This Journal*, 12, 310 (1929).
[7] *Analyst*, 55, 554 (1930).
[8] *J. Biol. Chem.*, 88, 347 (1930).
[9] *J. Ind. Eng. Chem.*, 6, 522 (1912).
[10] *Ind. J. Med. Research*, 16, 788 (1929).

To prevent the retention of manganese by silica the acid-insoluble residue was digested with hydrofluoric and sulfuric acids. The difficulties experienced by Gorter and Rost[1] while using this prodcedure on soils were not encountered as practically all the residue went into solution after the digestion.

TABLE 1.
Manganese in plant materials
(Percentage of Mn_3O_4 on air-dried basis.)

PLANT MATERIALS	METHODS		
	POTASSIUM PERIODATE	AMMONIUM PERSULFATE	SODIUM BISMUTHATE
Wheat	0.0073	0.0073	0.0070
Rye	0.0099	0.0099	—
Corn	0.0010	0.0010	0.00058
Rice	0.0014	0.0015	—
Oats	0.0067	0.0070	—
Wheat straw	0.0218	0.0209	0.0161
Lettuce	0.0209	0.0218	0.0161
Kale	0.0157	0.0160	—
Beet tops	0.0182	0.0167	0.0152
Broccoli	0.0051	0.0061	—

MANGANESE IN PLANT MATERIALS

Wheat, rye, corn, rice, oats, wheat straw, lettuce, kale, beet tops, and broccoli were ground in air-dried condition and analyzed for manganese by the periodate and persulfate methods. Some of these materials were also analyzed by the bismuthate method.

The results are given in Table 1. The determinations by the periodate and the persulfate methods are in close agreement, and in four out of five cases the determinations by the bismuthate method are much lower than those obtained by the other two methods. It also took a considerably longer time to develop the permanganate color with sodium bismuthate than with potassium periodate or ammonium persulfate. When allowed to stand for about 24 hours the permanganate color developed with potassium periodate and ammonium persulfate suffered no change, while the color developed with sodium bismuthate faded quite appreciably. The claim of Gortner and Rost[2] and others that oxidation with ammonium persulfate does not develop the true permanganate color was not substantiated by these results. Gortner and Rost obtained better results with sodium bismuthate than with ammonium persulfate. However, a close examination of their results shows that they were not comparing the relative merits of these two methods, but were comparing the methods for preventing retention of manganese by silica, and what they actually

[1] Loc. cit.
[2] Loc. cit

found was that the fusion with sodium carbonate used by them for this purpose is more suitable for soils than digestion with hydrofluoric and sulfuric acids used by Hillebrand[1] for the same purpose in rock analysis. When they repeated the digestion with hydrofluoric acid three times, their results obtained with the persulfate method were practically identical with those obtained with the bismuthate method.

TABLE 2.

Manganese in potassium permanganate and manganic oxide.

MANGANESE COMPOUNDS	ALIQUOT	THEORETICAL QUANTITY OF Mn_2O_4	POTASSIUM PERIODATE METHOD	Mn_2O_4 DETERMINED BY THE	
				AMMONIUM PERSULFATE METHOD	SODIUM BISMUTHATE METHOD
		mg.	*mg.*	*mg.*	*mg.*
Potassium Permanganate	1 cc. of 0.1 *N* solution	1.52	1.52	1.52	1.37
Manganic Oxide	2 mg.	1.93	1.98	1.98	1.67

The results in Table 1 show that the potassium periodate and ammonium persulfate methods are equally suitable for the determination of manganese in plant materials. Ammonium persulfate is much cheaper than potassium periodate. On the other hand, when the plant materials contain an appreciable quantity of chlorides the ash must be ignited with sulfuric acid to expel the chlorine if the persulfate method is used, while in the use of the periodate method chlorides (in contra-distinction from hydrochloric acid) do not interfere.

MANGANESE IN INORGANIC MANGANESE COMPOUNDS

Aliquots of 1 cc of 0.1 *N* potassium permanganate solution, to which enough sodium oxalate was added to destroy the pink color, and of 2 mg. of manganic oxide dissolved in nitric acid were evaporated, respectively, nearly to dryness and analyzed for manganese by the three methods. The results, given in Table 2, show again that identical and practically theoretical values for manganese were obtained by the periodate and persulfate methods, while those obtained with the bismuthate methods were considerably lower.

GRAVIMETRIC DETERMINATION OF MANGANESE IN PLANT MATERIALS

Wheat, rye, wheat straw, lettuce, and kale were analyzed gravimetrically for manganese. The results are given in Table 3. Column 1 of this table gives the manganese values obtained by the periodate method

[1] U. S. Geol. Survey Bull. 422 (1910).

(taken from Table 1); column 2 gives the gravimetric values and column 3 gives the values obtained by dissolving the gravimetric precipitates and oxidizing them with potassium periodate.

A comparison of the three columns of this table shows that the gravimetric values are much higher and their periodate values much lower than the values obtained by the potassium periodate method directly. Evi-

TABLE 3.

Determination of manganese in plant materials by the gravimetric and potassium periodate methods.

(*Percentage of Mn_3O_4 on air-dried basis.*)

PLANT MATERIALS	POTASSIUM PERIODATE METHOD	GRAVIMETRIC METHOD	MANGANESE IN GRAVIMETRIC PRECIPITATE DETERMINED BY POTASSIUM PERIODATE METHOD
Wheat	0.0073	0.022	0.0058
Rye	0.0099	0.031	0.0079
Wheat straw	0.0218	0.042	0.0130
Lettuce	0.0209	0.108	0.0184
Kale	0.0157	0.080	0.0151

dently foreign material was precipitated with the manganese, while the precipitation of the manganese itself was not complete.

The results demonstrate that the gravimetric method is not accurate for estimating small quantities of manganese in plant materials, thus corroborating the generally accepted view.

SUMMARY

Plant materials and inorganic manganese compounds were analyzed colorimetrically for manganese by the potassium periodate, ammonium persulfate and sodium bismuthate methods and also gravimetrically.

The potassium periodate and the ammonium persulfate methods were found to be equally suitable for the determination of manganese in plant materials. The sodium bismuthate method yielded results appreciably lower than those obtained by the other two colorimetric methods. The gravimetric method was found to be inaccurate for estimating manganese in plant materials.

THE DETERMINATION OF PLANT ASH CONSTITUENTS IN THE PRESENCE OF SILICA*

By JEHIEL DAVIDSON, (Food Research Division, U. S. Bureau of Chemistry and Soils).

In connection with an investigation in the Crop Chemistry Laboratory difficulties were encountered in obtaining satisfactory agreement between

*Food Research Division Contribution No. 111.

duplicate determinations of potassium in wheat straw. On volatilizing the silica from the ash with hydrofluoric acid good agreement between duplicate results was obtained, and the potassium results were appreciably higher than those obtained without silica volatilization.

The analysis of straw is important because it is used in feeds and is returned to the soil in manure and in ploughed-under stubble. A correct analysis is also essential in estimating the quantity of plant food removed from the soil by growing crops. It was therefore considered worthwhile to investigate fully the effect of silica on the recovery of potassium from straw ash, and also its effect on the recovery of the other common ash constituents,—phosphorus, calcium, magnesium, manganese, iron and aluminum.

MATERIALS

The materials selected for study were wheat straw of the "purple straw" variety, grown on the government farm at Arlington, Virginia, and two varieties of rice straw grown at Briggs, California.[1] The wheat straw was obtained from plots used for the Bureau's investigation of the relation of the lignin content to the loding of cereals.[2] One plot served as control and received no fertilizers; the other plot received sodium nitrate at the rate of 600 pounds per acre. The straw from the fertilized plot had an appreciably lower silica content than the straw from the control plot. The rice straws were obtained from pot experiments in which two varieties of rice, Coluza and Kama Irza were grown with and without a complete fertilizer (potassium, phosphorus, and nitrogen). The rice straw from the fertilized pots was likewise lower in silica than the straw from the control pots.

METHODS AND PROCEDURE

The procedure in preparing the solutions of the ash was as follows: (1) The ash was dissolved in hydrochloric acid (1+4) and filtered, and the filtrate was made up to volume and analyzed for the ash constituents. The residue and filter paper were returned to the electric muffle for ignition, weighed, digested on the steam bath with hydrofluoric and sulfuric acids, reignited, and again weighed and dissolved in dilute hydrochloric acid. The filtrate was made up to volume and again analyzed for the ash constituents. The second residue was again returned to the muffle and weighed. (2) The ash was digested with hydrofluoric and sulfuric acids, without previous removal of the acid-soluble ash. On volatilization of the silica, the residue was ignited, weighed, dissolved in dilute hydrochloric acid, filtered, and analyzed.

The acid-soluble ash was determined by difference in weight of the original ash before and after leaching with dilute hydrochloric acid, and

[1] The writer is indebted to J. W. Jones of the Bureau of Plant Industry for furnishing these samples.
[2] Davidson and Phillips, *Science*, 72, 401 (1930).

the silica by difference in weight before and after digestion with hydrofluoric and sulfuric acids. The determinations of the various ash constituents were made in accordance with the methods of the Association of Official Agricultural Chemists for Plants.[1] The phosphorus was determined volumetrically, the potassium as potassium chloroplatinate, the calcium by titration of the oxalate with standard permanganate, and the

TABLE 1.

Soluble ash in rice straw obtained by volatilizing silica with hydrofluoric and sulfuric acids and with hydrofluoric acid alone.

VARIETY	TREATMENT	ACID-SOLUBLE ASH	
		WITH HYDROFLUORIC AND SULFURIC ACIDS	WITH HYDROFLUORIC ACID ALONE
Coluza	Control	5.47	4.86
Kama Irza	Control	6.65	5.85
Coluza	Complete fertilizer	14.82	9.62
Kama Irza	Complete fertilizer	16.21	10.04

magnesium gravimetrically. Iron and aluminum were weighed together without determining the iron and aluminum separately, and manganese was determined colorimetrically.

RESULTS

Duplicate determinations resulted in fairly good agreement. In the majority of cases the figures in the tables are average percentages, calculated on the basis of the air-dried straw. Table 2 gives the analytical results for the wheat straw and Table 3 the results for the rice straw. The results of the silica and ash determinations are given in one column each, those of the acid-soluble ash in four columns, and those of each of the other determinables in five columns. Column 1 gives the average percentages of each constituent obtained by direct treatment of the ash with dilute hydrochloric acid and without volatilization of the silica; column 2 gives the percentages of each constituent found in the residue from the first acid treatment after volatilization of the silica; and column 3 in each case gives the total percentages obtained by adding the figures of columns 1 and 2. Column 4 gives in each case the total percentages obtained by directly digesting the whole ash with hydrofluoric and sulfuric acids and then dissolving it in hydrochloric acid, the figures being comparable with those of column 3. Column 5 gives the relative values of the figures of column 1 and of column 4, taking those of column 4 as 100. While the figures of columns 3 and 4 are in fair agreement, the direct totals (column 4) are considered more dependable than the added totals (column 3) in which unavoidable inaccuracies were multiplied by two. In the case of the acid-

[1] *Methods of Analysis*, 1925, p. 39.

TABLE 2.

Determination of ash constituents of wheat straw with and without volatilisation of silica.
(Percentage on air-dried basis.)

PART I

TREATMENT	ASH	SILICA	ACID-SOLUBLE ASH				P_2O_5					K_2O				
			1*	2	3	5	1	2	3	4	5	1	2	3	4	5
Control	5.95	5.07	1.32	.48	1.80	73	.174	.016	.190	.200	87	.315	.214	.529	.562	57
Sodium nitrate	5.03	2.48	1.92	.46	2.38	81	.140	.046	.186	.181	77	.383	.449	.832	.863	44

PART II

TREATMENT	CaO					MgO					Al_2O_3 and Fe_2O_3				
	1	2	3	4	5	1	2	3	4	5	1	2	3	4	5
Control	.258	.026	.284	.283	91	.90	.017	.107	.099	91	.096	.150	.246	.206+	47
Sodium nitrate	.236	.024	.260	.273	86	.107	.021	.128	.118	91	.040	.094	.134	.102	39

(1) Without volatilisation of silica.
(2) In acid-insoluble residue after volatilisation of silica.
(3) Added total = column 1 + column 2.
(4) Direct total—ash digested with hydrofluoric acid before being dissolved in hydrochloric acid.
(5) Percentage recovery: $\frac{\text{Column 1}}{\text{Column 4}} \times 100$. With acid-soluble ash Column 5 = $\frac{\text{Column 1}}{\text{Column 3}} \times 100$.

* One determination.

TABLE 3.

Ash constituents of rice straw determined with and without volatilization of silica.
(Percentage on air-dried basis.)

PART I

VARIETY	TREATMENT	ASH	SILICA	ACID-SOLUBLE ASH				P_2O_5					K_2O				
				(1)	(2)	(3)	(5)	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)
Caluss	Control	17.74	13.14	1.55	2.93	4.49	35	0.106	0.017	0.123	0.124	85	0.861	1.018	1.879	1.877	46
Kama Irsa	Control	16.25	11.69	3.16	1.57	4.74	67	0.140	0.015	0.155	0.150	93	2.136	0.461	2.597	2.558	83
Caluss	Complete fertiliser	15.84	6.35	5.64	3.73	9.36	60	0.194	0.001	0.195	0.199	97	0.763	0.298	1.061	1.003	76
Kama Irsa	Complete fertiliser	17.72	7.40	6.36	3.48	9.84	65	0.127	0.004	.0131	0.122	104	0.648	0.268	0.916	0.867	75

PART II

VARIETY	TREATMENT	CaO					MgO					Fe_2O_3 AND Al_2O_3					Mn_3O_4				
		(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)	(1)	(2)	(3)	(4)	(5)
Caluss	Control	0.075	0.149	0.224	0.243	31	0.146	0.136	0.282	0.288	51	0.064	0.026	0.090	0.082+	78	0.02₀	0.030	0.058	0.053	53
Kama Irsa	Control	0.154	0.071	0.225	0.258	60	0.193	0.078	0.271	0.265	73	0.068	0.047+	0.115	—	—	0.034	0.015	0.049	0.045	75
Caluss	Complete fertiliser	1.080	0.519	1.600	1.757	61	1.181	0.261	1.442	1.432	82	0.197	0.066	0.264	0.254	76	0.183	0.021	0.204	0.213	86
Kama Irsa	Complete fertiliser	0.967	0.557	1.525	2.009	48	1.240+	0.228	1.468	1.416	87	0.360	0.080	0.440	0.435+	83	0.183	0.017	0.200	0.213	86

(1) Without volatilisation of silica.
(2) In acid-insoluble residue after volatilisation of silica.
(3) Added total = column 1 + column 2.
(4) Direct total—ash digested with hydrofluoric acid before being dissolved in hydrochloric acid.
(5) Percentage recovery: $\frac{\text{Column 1}}{\text{Column 4}} \times 100$. With acid-soluble ash Column 5 = $\frac{\text{Column 1}}{\text{Column 3}} \times 100$.

* One determination.

soluble ash, the figures of column 5 were obtained by comparing column 1 with column 3, as column 4 was omitted in this case. The omission is due to the fact that when the ash is digested with hydrofluoric and sulfuric acids the soluble bases are converted into sulfates and as a result the figures for soluble ash are too high. This is clearly shown by Table 1.

In one case, as shown in Table 1, column 1, the straw was ashed and then digested with hydrofluoric and sulfuric acids; in the other case (column 2) the plant material was digested with hydrofluoric acid and then ashed without the addition of sulfuric acid. In the latter case the figures for soluble ash agree fairly well with those of column 3, Table 3, which are also somewhat too high owing to the fact that the portion of the bases retained by the acid-insoluble residue was subsequently converted into sulfates. In the former case they are much higher than the respective figures for total soluble ash in column 3, Table 3.

Using the same reasoning, it will follow that the silica figures, especially those of Table 3, are somewhat too low, as the conversion of the bases retained by the insoluble residue into sulfates reduces the apparent loss of silica by volatilization, which is determined by difference. It is apparent, therefore, that when the quantity of bases retained by the silica during hydrochloric acid digestion is appreciable, the analyst is faced with a dilemma—if he reports the insoluble residue as silica, the results will be too high, and if he digests the residue with hydrofluoric and especially with hydrofluoric and sulfuric acids and determines the silica by loss in weight, the results will be somewhat too low. The difficulty, however, may be solved by adopting Kuzirian's[1] modification, which consists in treating the acid-insoluble residue with sulfuric acid, igniting, and weighing it before the silica is volatilized with hydrofluoric and sulfuric acids.

The recovery of the ash constituents of the wheat straw from the hydrochloric acid solutions obtained without previous volatilization of silica was incomplete in every case. The lowest recovery was obtained in the case of potassium and iron and aluminum. The percentages of recovery were not related inversely to the silica content as might be expected. In the majority of cases, to the contrary, the highest recoveries were obtained from the straw from the unfertilized wheat plot, which had a higher silica content than the straw from the fertilized plot.

The recovery of ash constituents of the rice straw from the hydrochloric acid solutions without previous volatilization of silica was likewise incomplete except in the case of phosphorus, especially in the straw from the fertilized pots. The Coluza straw grown without fertilizers gave the poorest recovery in the case of every constituent. Of the individual ash constituents, the poorest recovery was in the case of calcium. As with the wheat straw, the results for the rice straw do not indicate any inverse relation between recovery and silica content. However, a comparison of columns 2 and 4 (3 for the acid-soluble ash) shows that the absolute re-

[1] *Am. J. Science*, 37, 61 (1914).

tention of ash constituents by the acid-insoluble residue is on the whole related to the total quantities present. This fact is most clearly brought out by comparing the retention figures for potassium (column 2) in both wheat and rice straw (Tables 2 and 3). The fertilizer treatment of the wheat straw resulted in an increased potassium content; with rice straw it resulted in a decreased potassium content; but in both cases the greater potassium content coincided with a greater retention of this element by the acid-insoluble residue.

It is clear from these results that an analysis of the ash constituents of straw and most likely of other plant substances relatively high in silica is incomplete unless precautions are taken against retention of the ash constituent by the acid-insoluble residue.

DISCUSSION

The results of this investigation indicate the importance of acid-soluble ash determinations in the analysis of feeds and fodders which are frequently rich in silica. Generally in an incomplete analysis of these substances, the mineral analysis is limited to the determination of the ash, which is assumed to be the sum total of the nutritive elements, phosphorus, potassium, calcium, magnesium, etc. But when results for the total ash are given they also include silica, which is not considered an essential element of nutrition, although it is always present in human and animal tissue.[1] In such cases, therefore, it is obvious that the total ash determination should be supplemented by determinations of acid-soluble ash.

The food or fertilizer value of ash constituents retained by the acid-insoluble residue is of course not known. The question deserves investigation. It is possible that the portion of the ash which is insoluble in dilute hydrochloric acid previous to volatilization of silica is not available either to plants or animals. However, in estimating the plant food removed from the soil by crops this portion must always be considered.

The mechanism by which a soluble portion of ash constituents is held in the acid-insoluble residue cannot be determined from the data at hand. The fact that the variations in silica content had no effect on the magnitude of retention does not point to adsorption. On the other hand, the fact that retention tended to increase with the concentration of the acid-soluble ash constituents is in agreement with adsorption phenomena. It is possible that the retained bases are in the form of insoluble silicates and that their formation is related to their total quantities present in the ash. If such were the case, the error in analysis could also be avoided by fusing the ash with alkalies at high temperatures.[2] It would then be necessary to

[1] *Z. physiol. Chem.*, 194, 81 (1931).

[2] Since this article was prepared for publication, there appeared in the analytical edition of *Ind. Eng. Chem*, 3, 164 (1931), an article by H. P. Morris, J. W. Nelson, and L. S. Palmer, who also found that the acid-insoluble ash interfered with the proper determination of calcium, magnesium, and phosphorus in foodstuffs and cattle excreta. While using colorimetric methods they found that fusion of the ash with sodium carbonate was more satisfactory in preventing retention of ash constituents by the acid-insoluble residue than volatilisation with hydrofluoric acid alone. In the present investigation, however, the ash was digested with hydrofluoric and sulfuric acids.

make the determination of silica and other ash constituents according to the procedure used in the analysis of silicates. The retention of bases is so small under these conditions that it may be neglected in plant analysis.[1] However, when the determination of both sodium and potassium in the plant ash is wanted the silica, when present in relatively large quantities, will have to be volatilized with hydrofluoric acid in order to obtain complete recovery of the ash constituents.

The subject deserves further study.

SUMMARY

Wheat and rice straws grown with and without fertilizers were analyzed for total and acid-soluble ash, silica, phosphorus, potassium, calcium, magnesium, iron and aluminum, and manganese.

When the ash of wheat and rice straw was dissolved with dilute hydrochloric acid without previous volatilization of silica, the results of analysis in every case were too low. When the acid-insoluble residue was digested with hydrofluoric and sulfuric acids to expel the silica and then redissolved in dilute hydrochloric acid it yielded additional quantities of the ash constituents.

When the ash was digested directly with hydrofluoric and sulfuric acids and then dissolved in dilute hydrochoric acid the results agreed with the sums of the respective results obtained from the solutions of the ash and the insoluble residue after digestion with hydrofluoric and sulfuric acids except in the case of the acid-soluble ash and silica. The exception in the last two cases is due to the conversion of the bases into sulfates, rendering the results for acid-soluble ash too high and those for silica too low.

Volatilization of silica or possibly other procedures to prevent retention of bases by the acid-insoluble residue is essential to the proper analysis of ash constituents of plant substances rich in silica.

It is recommended that the acid-soluble ash in plant substances rich in silica be determined as well as the total ash when analyses of the ash constituents are omitted.

A RAPID METHOD FOR DETERMINING ACID-SOLUBLE PHOSPHORIC ACID IN EGGS

By J. FITELSON and I. A. GAINES, JR. (New York Station, Food and Drug Administration, U. S. Department of Agriculture).

INTRODUCTION

Chapin and Powick[2] studied the ratio between the inorganic and total phosphorus as an index of decomposition of eggs. Pine[3] considers the

[1] Bloor, W. R., *J. Am. Chem. Soc.*, 29, 1603 (1907); U. S. Geol. Survey Bull. 422, p. 97 (1910).
[2] *J. Biol. Chem.*, 20, 97 (1915).
[3] *This Journal*, 8, 57 (1924).

acid-soluble phosphoric acid a better index and gives experimental verification of his analytical method. Macomber[1] attempted to shorten Pine's method as applied to liquid and dried eggs.

The work reported here was undertaken to devise a rapid modification of Pine's method, with particular reference to its use in the determination of decomposition in dried egg yolk. Analyses of dried whole egg, liquid yolk, and liquid whole egg were also made to compare the proposed method with the Pine method.

TABLE 1.

Comparison of extraction methods.

SAMPLE	PINE'S METHOD ($Mg.P_2O_5$ PER 100 GRAMS, DRY BASIS)	PROPOSED METHOD
B.B.W. (dried yolk)	137.0	135.8
20698 (dried yolk)	136.1	137.7
S.C. (dried yolk)	111.7	109.9

Pine's method may be separated into three procedures: (a) extraction of the acid-soluble phosphoric acid; (b) destruction of the organic matter in the extract; and (c) estimation of the phosphoric acid. Any changes in these procedures should give results concordant with those obtained by the original method.

EXPERIMENTAL WORK

(*a*) *Extraction.*—Pine weighs the egg into an Erlenmeyer flask, adds 200 cc. of dilute hydrochloric acid and 8 grams of picric acid, stoppers, and shakes at 10 minute intervals for 1 hour. He then filters the mixture, allowing the egg to remain in contact with the extracting medium not exceeding 1 ¾ hours. Pine has also shown that shaking by machine for ½ hour is equivalent to the 1 hour shaking by hand. However, if the extraction is continued for more than 1 hour an increased yield of phosphoric acid is obtained.

Macomber hastens the filtration of the extract by centrifugalizing. It was thought that the use of a hydrochloric acid-picric acid solution would simplify the manipulation somewhat, especially when several samples are analyzed at the same time, and also eliminate the correction applied by Pine for the moisture in the picric acid. An additional pinch of picric acid insures a saturated picric acid solution. The modified procedure makes use of the hydrochloric acid-picric acid solution, the shaking machine, and the centrifuge.

Several experiments were conducted to compare the results obtained by the modified and the original extraction methods. Table 1 indicates that the modified extraction gives practically the same results as Pine's method. The general correlation of the results given in Table 4 also indicates that the two extraction methods give comparable results.

[1] *This Journal*, 8, 604 (1925); 12, 351 (1929).

(b) *Destruction of Organic Matter.*—Since the volumetric estimation of phosphoric acid is more rapid than the gravimetric method, attempts were made to modify this second step in the determination so that a final volumetric estimation could be used. Pine's use of 10 cc. of sulfuric acid together with nitric acid precludes the use of the usual volumetric method involving precipitation with molybdate at 45–50°C.[1]

Any method proposed for the destruction of organic matter must convert the organically combined phosphorus into an inorganic form suitable

TABLE 2.

Oxidation of glycerophosphoric acid.

METHOD	P_2O_5 (mg. per 100 cc.) GRAVIMETRIC	VOLUMETRIC
1	37.8	—
2	36.4	39.5
3	—	20.7
4	—	28.5
5	—	37.8

for estimation. It was also necessary to show that the destruction of organic matter was complete. To eliminate any uncertainty of extraction a solution of pure glycerophosphoric acid[2] was used in the initial experimental work.

The following methods and reagents were tried to compare their usefulness: (1) sulfuric and nitric acids; (2) ignition with excess alkali; (3) concentrated nitric acid and superoxyl; (4) aqua regia followed by acid permanganate; (5) aqua regia followed by alkaline permanganate.

The results in Table 2 show that methods (2) and (5) convert glycerophosphoric acid as completely as does the sulfuric-nitric combination. However, the latter is more easily manipulated.

Macomber found that 5 cc. of sulfuric acid with sufficient nitric acid to oxidize organic matter were "fully as effective in the destruction of organic matter as the 10–20 cc. combination." This conclusion was verified by the writers on several samples of egg extract. It was therefore decided to adopt Macomber's modification of this procedure for the destruction of organic matter in egg extract as this might also permit the use of a final volumetric estimation.

(c) *Estimation of Phosphoric Acid.*—Ross[3] has shown that by precipitation at room temperature with continuous stirring phosphoric acid may be accurately determined volumetrically in the presence of sulfates equivalent to 2–3 cc. of concentrated sulfuric acid. It is apparent that if egg extract is digested with 5 cc. of sulfuric acid, an appreciable portion of the acid will be used up. Nevertheless, it was considered advisable to investigate the effect of the maximum quantity (5 cc.) on the volumetric esti-

[1] *Methods of Analysis, A.O.A.C.*, 1925, 3, 4.
[2] *This Journal*, 8, 57 (1924).
[3] *Ibid.*, 13, 203 (1930).

mation of phosphoric acid under the above conditions. A pure phosphate solution was used. Table 3 gives the results. These show that the presence of sulfates equivalent to 5 cc. of concentrated sulfuric acid cause only slightly high results. The difference noted would affect the ordinary result on eggs by about 1 mg. per 100 grams. This difference is well within the usual accuracy of the entire determination, based on the writers' experience.

TABLE 3.

Volumetric determination of P_2O_5.

P_2O_5 (mg. per 100 cc.)

ANALYST	SULFATES ABSENT, PRECIPITATION AT 45°C.	SULFATES PRESENT, PRECIPITATION AT 25°–30°C. WITH STIRRING
J.F.	16.06	16.24
I.A.G. Jr.	16.05	16.26
	16.12	16.19
Average	16.08	16.23

PROPOSED METHOD

The preceding considerations led to the following proposed rapid method:

SOLUTION USED

Picric acid solution.—Saturated. Immediately before use add 0.5 cc. of concentrated hydrochloric acid to each 100 cc.

PROCEDURE

Weigh 12 grams of dried egg yolk (25 grams of dried whole egg, 50 grams of liquid whole egg, or 25 grams of liquid yolk) and transfer to an 8 oz. centrifuge bottle. Add exactly 150 cc. of the freshly made picric acid solution and a pinch of picric acid to insure saturation. Stopper, shake vigorously for a few minutes, place on a mixing machine for 30 minutes, centrifugalize, and filter through a folded filter paper. (The egg material should not remain in contact with the acid solution for more than 1¾ hours, including the time required for filtration.)

Pipet 100 cc. of the filtrate into a 300 cc. Kjeldahl flask and add a few glass beads, 5 cc. of concentrated sulfuric acid, and 20 cc. of concentrated nitric acid. Boil until white fumes appear. Add nitric acid in small quantities until a practically colorless solution is obtained when the white fumes appear. Continue boiling for 30 minutes, cool, and wash the acid solution into a 400 cc. beaker, keeping the total volume below 100 cc.

Add 10 grams of ammonium nitrate and then make alkaline to litmus with strong ammonia. Acidify slightly with nitric acid, cool to 25–30°C., place in a stirring apparatus, and add 45 cc. of the volumetric molybdate solution.[1] Stir continuously for at least 30 minutes, filter, wash, and complete the determination as directed under 10 (a).[1]

Correct the volume of the extraction mixture for the water in the eggs. Report the phosphoric acid as mg. P_2O_5 per 100 grams of *dry* egg.

To verify the accuracy of the proposed method, several samples were run both by it and by Pine's method. The data given in Table 4 show good

[1] *Methods of Analysis, A.O.A.C.*, 1925, 3, 8(a).

agreement between the two methods on dried yolk and whole egg, and on liquid yolk and whole egg.

SUMMARY

Pine's method (2) for the determination of acid-soluble phosphoric acid in eggs was modified as suggested in the literature and according to the experimental work reported in this paper. The modified method involves (a) the use of 5 cc. of concentrated sulfuric acid and sufficient nitric acid to destroy organic matter in the extract from the egg; (b) the volumetric estimation of phosphoric acid after precipitating at 25–30°C.; and (c) certain modifications in the extraction of the phosphoric acid. The method gives results which are comparable to those secured by the method used by Pine and requires considerably less elapsed time.

The authors wish to acknowledge their indebtedness to H. D. Grigsby, New York Station, U. S. Food and Drug Administration, for his encouragement and cooperation in the preparation of this paper.

TABLE 4.

Comparison of Pine's method and the proposed rapid method for the determination of acid-soluble phosphoric acid in egg.

SAMPLE	ANALYST	PINE'S METHOD (MG. P_2O_5 PER 100 GRAMS, DRY BASIS)	PROPOSED METHOD (MG. P_2O_5 PER 100 GRAMS, DRY BASIS)
Dried egg yolk			
B.B.W.	J.F.	133.5	134.3
	I.A.G	136.5	137.3
21026	J.F.	140.5 139.5	133.0
	I.A.G.	134.5	135.8
20526	J.F.	145.8	146.2
	I.A.G.	140.2	145.5
S.C.	J.F.	119.6	113.6
	I.A.G.	109.6	115.2
20698	J.F.	136.1	135.5
	I.A.G.	137.7	134.4
Dried whole egg	J.F.	106.0	112.1
	I.A.G.	114.8	113.6
Liquid whole egg	J.F.	—	90.6
	I.A.G.	99.3	92.5
Liquid egg yolk	J.F.	119.6	113.2
	I.A.G.	110.8	114.8

A STATISTICAL STUDY OF SOME SAMPLING RELATIONS WITH SPECIAL REFERENCE TO QUANTITATIVE MICROSCOPY

By J. D. WILDMAN (Microanalytical Laboratory, U. S. Food and Drug Administration, Washington, D. C.).

It is the purpose of this paper to indicate the application to certain sampling problems of a useful statistical formula. The material presented can not be considered entirely original inasmuch as the sampling relation to be considered has been known for some time, and although it has found numerous applications in various fields, its importance in sampling problems with which the official analyst has to deal has not been generally recognized.

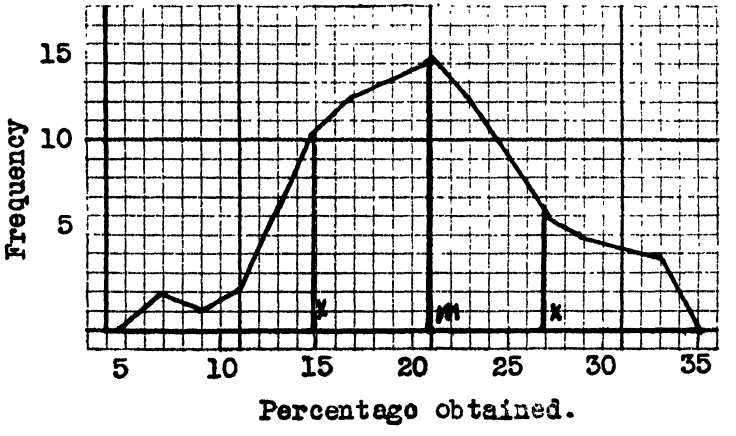


FIG. 1.—*Frequency distribution of results of testing 100 samples of 50 nuts each.*
M = line erected at mean or average.
X = line erected at distance of 1σ from the mean.

That successive analyses made on the same sample occasionally appear to yield discordant results is well known. This is more particularly true when analyses are made on mixtures rather than upon solutions. The present discussion is confined to mixtures and especially to those materials which, in a regulatory sense, consist of both passable and nonpassable units, or in general of positive and negative units. For example, if 100 successive samples of 50 nuts each are drawn from the same bag and cracked and the percentage of wormy nuts is calculated for each sample, it will be found that the results will fluctuate rather widely around the average value. If it were possible to remove all variation due to the analyst, to the method, or to a lack of representativeness, it would be found that there still remains considerable variation in the series of analyses. This remaining fluctuation, which may be called sample variation, is the subject under discussion.

For the sake of clearness some of the terms used in describing frequency distributions will be mentioned first. As an illustration the results on the sampling of nuts recorded by B. J. Howard[1] of this laboratory, may be cited. In one of the experiments 400 nuts were marked so as to be identifiable and then thoroughly mixed with 1600 unmarked nuts. Successive samples were then drawn, and the percentages of marked nuts were determined until a total of 100 samples of 50 nuts each had been tested. Fig. 1 shows these results when plotted on a chart in which the vertical axis represents numbers of samples and the horizontal axis represents percentages.

It will be noticed in Fig. 1 that three lines have been drawn perpendicular to the base, the center one (M) being drawn at the average or mean of the 100 samples, or 20.88 per cent. The two lines (X, X) on either side of the mean enclose an area which is roughly 68 per cent of the total area beneath the curve (i.e., approximately 68 per cent of all the percentages obtained from the successive determinations fall between the two lines). The distance from the mean line to either of the other lines is denoted by the symbol σ, representing the standard deviation. In this particular instance the standard deviation amounts to 6.1 per cent, which means simply that about two-thirds (actually 68.3 per cent) of the 100 analyses made fall within the limits of 6.1 per cent either side of the average, or, in other words, within the range from 14.78 per cent to 26.89 per cent. The standard deviation has been found to be a suitable means of measuring variability. The total range has been used at times to describe the fluctuation in results, but this is not a reliable practice as the limits depend upon only a few observations, whereas the standard deviation is based upon the whole population.

In brief, the standard deviation is a single figure which expresses the degree of dispersion obtained in any particular frequency distribution. In practice it is not feasible to make a large number of determinations on a single sample for the purpose of determining the amount of variation to be expected. The best that can be done for some products is to make a series of analyses on the same material and from the results calculate a standard deviation which can be used as a rough guide for future determinations. An example of this type of procedure is offered by the work of Barnes[2] on arsenic determinations.

For certain materials, namely those that consist of a mixture of passable and nonpassable units, such as nuts, figs, cocoa beans, etc., the procedure is somewhat simplified. With such products the results are expressed as a percentage of nonpassable units, from which the standard deviation can be calculated directly, that is without recourse to an expensive sampling program, by the use of the following formula:

[1] Unpublished data.
[2] *Ind. Eng. Chem.*, 21, 172 (1929).

Formula I

$$\sigma = \sqrt{\frac{PQ}{n}}$$

in which

σ = standard deviation
P = percentage of passable or positive units
$Q = 100 - P$
n = size of sample in units from which P is calculated. When n is less than 30, $n - 1$ should be used.

APPLICATION OF FORMULA

An example of the application to which the formula can be put is offered by the nut sampling data previously cited. The true percentage of marked nuts in this case was 20 per cent, so that P = 20 and Q = 80. Since each sample consisted of 50 nuts, n = 50. Substituting these values in the formula it is found that the true standard deviation for the problem is 5.657 per cent. That is, it may be expected from theoretical grounds that approximately 68 per cent of all 50-nut samples taken from a large number of nuts, 20 per cent of which were marked, will fall, in percentages, within a range from 14.34 per cent (20 per cent–5.657 per cent) to 25.66 per cent (20 per cent+5.657 per cent). In the actual experiment it was found that approximately 66 per cent of the samples fell between these limits. From this it is seen that this formula affords a ready means of determining approximately the degree of dispersion that would prevail in the kind of sampling problem described, were it possible to take an infinite number of subsamples from the same material. Various other essential relations can also be determined by simple calculation and tables, the presentation of which would lead too far afield for the present article. Collins[1] has called attention to several applications of the method and those interested should consult his paper.

APPLICATION TO MICROSCOPIC DATA

It was believed by the writer that a study of the applicability of the formula to quantitative microscopy might be of interest. In this field the units of which the mixtures in question are composed are very small; therefore, in order to determine whether or not it is permissible to apply the formula to certain microscopical data, it was decided to make a series of counts on different materials and then compare the actual standard deviations obtained with the standard deviation calculated from the formula. The results obtained follow.

Mixtures of Lycopodium Spores and Arrowroot Starch Grains

These two substances were chosen because both consist of microscopic elements which are easily distinguishable. Any error due to the analyst is thereby greatly reduced, as there is little likelihood of the lycopodium

[1] U. S. Dept. Agr. Circ. 79 (1929).

spores being confused with the starch grains. The first series of counts was made on a mixture made up approximately of equal numbers of lycopodium spores and starch grains. This mixture was obtained by adding

TABLE 1.

Illustration of method of calculating the actual standard deviation. Data consist of the percentages of lycopodium spores in a mixture containing arrowroot starch and lycopodium powder, each percentage being based upon 100 units counted.

%	f	d'	fd'	$f(d')^2$
64	1	−10	−10	100
65	1	−9	−9	81
66	2	−8	−16	128
67	1	−7	−7	49
68	2	−6	−12	72
69	2	−5	−10	50
70	4	−4	−16	64
71	6	−3	−18	54
72	15	−2	−30	60
73	10	−1	−10	10
74	12	0	0	0
75	10	1	10	10
76	8	2	16	32
77	8	3	24	72
78	5	4	20	80
79	5	5	25	125
80	3	6	18	108
81	1	7	7	49
82	2	8	16	128
83	0	9	0	0
84	1	10	10	100
85	1	11	11	121
Sum			+157	1493
			−138	
			+19	

$$c = \frac{\Sigma f d'}{n} = \frac{+19}{100} = +0.19$$

$$c^2 = 0.036$$

$$\sigma = \sqrt{\frac{f(d')^2}{n} = c^2} = \sqrt{\frac{1493}{100} - 0.036} = 3.86\%.$$

1 cc. of a 1 per cent suspension of lycopodium powder in mineral oil to 9 cc. of a 1 per cent suspension of arrowroot starch in mineral oil. The final suspension was well mixed in a small mortar, and drops were mounted on ordinary glass slides. For the second series of counts the percentage of lycopodium spores was increased by adding 1.5 cc. of the 1 per cent lycopodium suspension to the above suspension. Counts on this ma-

terial averaged around 74 per cent of lycopodium spores. Counts were made in the following manner, with a magnification of 90 diameters:

As the slide was moved, by means of a mechanical stage, all spores and starch grains which passed under a short line (0.23 mm. in length) etched on an ocular micrometer were counted. The results were recorded as the number of lycopodium spores per sample of 25 units of both lycopodium and starch, and the percentages were calculated.

TABLE 2.

Comparison of actual and theoretical standard deviations for mixtures of lycopodium powder and arrowroot starch.

STANDARD DEVIATION	SIZE OF SAMPLE TOTAL NUMBER OF UNITS 25	50	100	200
	per cent	*per cent*	*per cent*	*per cent*
Where P = approximately 52.4% of lycopodium spores by number				
Actual	10.87	6.98	4.97	3.67
Theoretical	9.99	7.05	4.99	3.53
Where P = approximately 74.8% of lycopodium spores by number				
Actual	8.68	5.92	3.86	2.60
Theoretical	8.66	6.17	4.37	3.09

Reference to the formula shows that as n increases σ decreases; i.e., as the number of units per sample is increased, the standard deviation is decreased. In order to increase the size of n experimentally, the original series of counts were combined by chance into counts of 50, 100 and 200 units, and the actual standard deviation for each distribution was calculated. In calculating the actual standard deviation, the usual formula was employed, namely,

Formula II

$$\sigma = \sqrt{\frac{\Sigma f(d')^2}{n} - c^2},$$

in which σ = standard deviation
f = frequency in any one class
d' = deviation from arbitrary or assumed mean
n = number of subsamples
c = correction factor $= \frac{\Sigma f d'}{n}$
Σ = summation

For those who are unfamiliar with the use of the above formula, a sample calculation is given in Table 1, the data obtained on the mixture of lycopodium spores and starch grains being used. The data in this particular series consist of the percentage by number of lycopodium spores in samples of 100 grains of both elements. One hundred such samples were counted, and the percentages were found to range from 64 to 85 per cent. This range is tabled in the column headed "%". In the column

headed "f" is given the frequency or number of samples having any particular percentage. The plus and minus values in column d' represent deviations from an arbitrary average, in this case 74 per cent.

In Table 2 the standard deviations, as calculated from the actual data, are compared with the theoretical standard deviations, as calculated by the use of Formula I.

TABLE 3.

Comparison of the actual and the theoretical standard deviations for mixtures of rag and chemical wood fibers.

STANDARD DEVIATION	SIZE OF SAMPLE TOTAL NUMBER OF FIBERS 25	50	100	200
	per cent	*per cent*	*per cent*	*per cent*
	Where P = approximately 48.3% of rag fiber			
Actual	9.39	6.76	4.33	3.12
Theoretical	9.99	7.07	4.99	3.53
	Where P = approximately 5.4% of rag fiber			
Actual	4.35	2.95	2.09	1.52
Theoretical	4.52	3.27	2.54	1.65

The agreement between the theoretical and actual results is obviously reasonably close.

PAPER FIBER COUNTS

Another study of the application of the formula was made in the paper stock count method in which the composition of paper stock is determined by counting the kinds of fibers in successive microscopic fields. To eliminate, so far as possible, any error due to the analyst, counts were made upon two mixtures of rag fibers and chemical wood fibers, which are rather easily distinguished by their color reaction when mounted in chlorzinciodide. The procedure given by Reed and Machman in their work "Determination of the Fiber Content of Paper"[1] was followed in making up the slide, with the exception that after the pulp had been well disintegrated and suspended in water, about 5 cc. of the suspension was placed in centrifuge tubes and centrifugalized until it was possible to pour off most of the liquid. About 2 cc. of chlorzinciodide was added to the tube and well mixed with the pulp by means of a pipet. The stained fibers were then transferred with the pipet to a slide, and the usual procedure was followed. Counts were made as before and results expressed as percentage of rag fibers. The results are given in Table 3.

COMBINATIONS OF POSITIVE AND NEGATIVE FIELDS

The figures given in Tables 2 and 3 show that the theoretical and actual figures check fairly closely. According to these experiments it would appear justifiable to utilize Formula I in microscopic work in which one

[1] Pub. by U. S. Gov. Printing Office (1923).

element is counted against another. However, in certain other methods one microscopic field is counted against another, i.e., if the field contains one or more of the elements being estimated, it is called positive, and the percentage of positive fields is used as an index of the total amount of the substance present. In order to determine whether the formula also holds for such methods the following procedure was used:

TABLE 4.

Comparison of the actual and the theoretical standard deviations for combinations of positive and negative fields.

STANDARD DEVIATION	SIZE OF SAMPLE TOTAL NUMBER OF FIELDS 25	50	100	200
	per cent	*per cent*	*per cent*	*per cent*
	Where P = approximately 13.9% of positive fields			
Actual	6.84	4.66	3.52	2.33
Theoretical	6.95	4.9	3.45	2.44
	Where P = approximately 67.5% of positive fields			
Actual	8.82	6.42	4.54	3.64
Theoretical	9.37	6.60	4.62	3.30

A small quantity of lycopodium powder (0.2 gram) was mixed with 600 cc. of tomato purée by means of an electric mixer. In order to be certain that each microscopic field represented the same volume of purée, a Howard counting cell was used for mounting the material. In counting, each field containing one or more of the lycopodium spores was recorded as positive. The fields were counted in groups of 25 fields each and the percentage of positive fields calculated therefrom; 100 such groups were counted in all. The original data were then combined by chance into samples of 50, 100, and 200 fields. The results are given in Table 4.

DISCUSSION

The tables showing the comparison between actual and theoretical values for the standard deviation indicate that the simple sampling relation under discussion can be used for determining the amount of sampling error to be expected in certain types of microscopic counts. This is further substantiated by the fact that no significant difference is found between the actual and the theoretical values for σ, when the two sets of data are compared by means of Pearson's χ^2 test,[1] a statistical method in rather wide use for comparing certain types of data. The agreement between the actual and theoretical data is well shown by Fig. 2, in which the actual values for σ are plotted on the vertical axis and the theoretical values on the horizontal axis.

[1] A discussion of the χ^2 method may be found in "Statistical Methods for Research Workers" by R. A. Fisher.

The opinion is sometimes expressed that owing to the smallness of sample used microscopical methods in general are less valuable than other methods in which larger quantities of material are used. From the results of the experiments and the known fundamental relations, how-

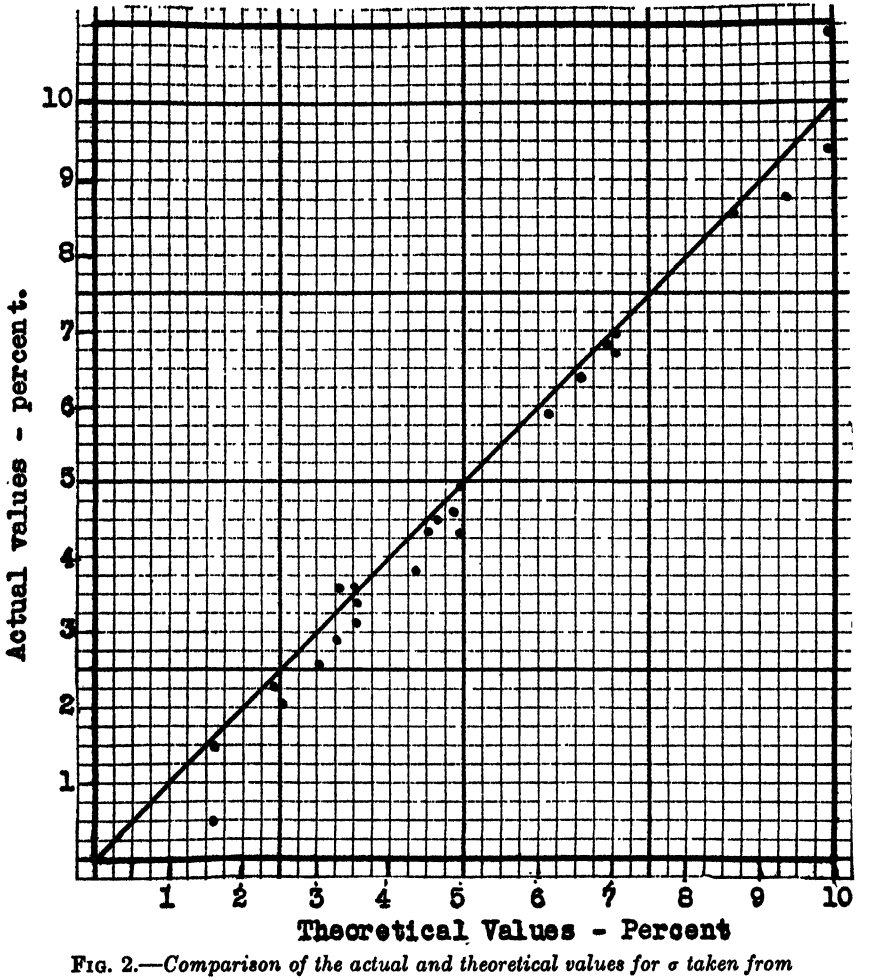


FIG. 2.—*Comparison of the actual and theoretical values for σ taken from Tables 2, 3, and 4.*

ever, it is obvious that the size of the *sample error* in microscopic methods of the type herein described is no greater than in macroscopic methods of the same type, and that there is no more reason for rejecting the results of microscopic examinations on the basis of sample error than there is of rejecting the results of the examination of much larger units. Moreover, the value of the resulting analysis in either case is greatly enhanced by a knowledge of the sample error present.

ESTERS AS ADULTERANTS OF CASSIA OIL, AND THEIR DETECTION

By JOSEPH CALLAWAY, JR., and THOMPSON N. BENNETT (U. S. Food and Drug Administration, New York, N. Y.)

Cinnamon oil, or cassia oil as it is more commonly called, is defined in U.S.P.X. as the volatile oil distilled from the leaves and twigs of *Cinnamomum Cassia* (Linné) Blume (Fam. *Lauraceae*), rectified by steam distillation.

It is specified that the oil shall contain at least 80 per cent by volume of cinnamic aldehyde, as determined by the neutral sodium sulfite method, and limits are set for the more important physical constants. Tests for such common adulterants as heavy metals, rosin or rosin oil, and chlorinated products are also outlined. The presence of the last-named would indicate the addition of synthetic cinnamic aldehyde.

In carrying out the U.S.P.X. assay method for cinnamic aldehyde, the authors found it desirable to use a saturated solution of sodium bisulfite instead of the 5 per cent solution as provided. The quantity of the 5 per cent solution necessary to maintain neutrality is so great that the total volume exceeds 100 cc. If a saturated (of nearly so) solution of sodium bisulfite is used, this difficulty is obviated.

In the examination of the crude oils from China, the tests for rosin or rosin oil and heavy metals have been found reasonably satisfactory. However, as synthetic cinnamic aldehyde, free from chlorine, is now available in European markets at a price lower than that of the oil, it is considered that a more reliable test for this product is needed.

Moreover, in dealing with redistilled oils during the last three years, several other adulterants which are not detected by the pharmacopeial tests have been noted. A simple test described later has been used for one type of these adulterants.

The most comprehensive published work on cassia oil with which the authors are familiar is that of Dodge.[1] He summarizes the knowledge of the constituents of the oil as follows:

Cinnamic aldehyde, 75–90 per cent
Cinnamyl acetate
Phenyl propyl acetate (?)
Methyl ortho-coumaric aldehyde
Salicylaldehyde, 0.1–0.2 per cent
Coumarin
Benzoic acid
Salicylic acid
Liquid acid of fruity odor
Benzaldehyde
Methyl salicylaldehyde

[1] *Ind. Eng. Chem.*, 10, 1005 (1918).

Assuming, then, an adulteration of the oil with synthetic cinnamic aldehyde, it seemed that this would necessitate an addition of nonaldehydes sufficient to render the proportion of cinnamic aldehyde normal. Therefore the nonaldehyde portion was further investigated.

A sample of mixed crude oils from China was distilled in the laboratory at atmospheric pressure. Several samples of commercially redistilled oils were procured from reliable sources, and the nonaldehyde portion was separated from 200 cc. of each oil. This was done by the U.S.P. assay method, as modified, by using liter flasks and pipetting off the nonaldehyde residue, drying it with anhydrous sodium sulfate, and filtering. These portions were fractioned at atmospheric pressure, and a saponification value, calculated as percentage of cinnamyl acetate, was obtained on each fraction. A rather representative summary follows:

FRACTION	COMMERCIALLY REDISTILLED		LABORATORY REDISTILLED	
	RECOVERED	ESTERS	RECOVERED	ESTERS
°C.	*per cent*	*per cent*	*per cent*	*per cent*
Below 200	10	18	26*	10
200–230	18	28	15	22
230–250	18	36	16	47
250–270	32	60	16	46
270–300	10	32	8	31

* Adulteration of Chinese crude oil probably accounts for high percentage recovered below 200°.

The results given tend to confirm the presence of cinnamyl acetate as the principal ester since it boils at about 257°C., and it was in fractions near this point that the maximum percentage of esters was obtained.

It was decided to test several crude oils for esters other than cinnamyl acetate. A well-recognized test for esters of the type wherein an alcohol is combined with an acid whose potassium salt is insoluble in strong alcohol, is to boil a small amount of the product with a 10 per cent solution of KOH in absolute alcohol. Since in the routine U.S.P. assay method for cinnamic aldehyde there is available about 1.5 cc. of nonaldehyde, the following procedure was adopted:

One cc. of the clear nonaldehyde residue was drawn off, after the completion of the U.S.P. assay for cinnamic aldehyde, and transferred to a test tube. About 3 cc. of a 10 per cent solution of KOH in absolute alcohol was added, and the mixture was boiled on the water bath for 2 or 3 minutes and cooled. The presence of foreign esters, of the type indicated above, was revealed by a precipitation which, in most cases, amounted to almost complete solidification of the mass.

In carrying out this test on a large number of crude oils, negative results were invariably obtained, while several samples of commercially redistilled oils gave positive results. Control samples consisting of the crude oil with the addition of about 5 per cent of benzyl benzoate and diethylphthalate, respectively, yielded heavy precipitates.

In the subsequent examinations of several commercial samples of the redistilled oil yielding positive tests, the precipitate obtained was filtered off, washed with a little absolute alcohol and ether to remove the remaining nonaldehyde, and then dissolved in water. This solution was acidified with dilute hydrochloric acid, the liberated acid was extracted with ether and the ether was evaporated. In each case the crystalline residues obtained were sublimed, and melting points were determined. In this manner esters of benzoic and phthalic acids were identified.

Although benzoic and salicylic acids have been reported as constituents of cassia oil, it is believed that they could not interfere with this test because they would be dissolved in the aqueous solution, probably as sodium salt, during the treatment with neutral sulfite.

SUMMARY

(1) The present official method for determining cinnamic aldehyde should be modified to provide for the use of a saturated solution of sodium bisulfite.

(2) The U.S.P. tests for the detection of adulterants in cassia oil fail to indicate the presence of chlorine-free synthetic cinnamic aldehyde or of foreign esters.

(3) The simple test described for one type of foreign esters has been used successfully for some time as a routine procedure.

NOTES

A Rapid Colorimetric Method for the Determination of Potassium by the Use of Cobaltinitrite*

In making a study of the quantities of potassium in plant extracts it was found almost impossible to use standard methods, when hundreds of determinations were desired, on account of the time and reagents consumed. Even the colorimetric method of Hill[1] was too time consuming.

In 1900 Aide and Wood[2] used sodium cobaltinitrite successfully in the gravimetric determination of potassium. However, this method also requires too much time.

The author found that by measuring colorimetrically the change in strength of the precipitating reagent, instead of making quantitative separations of the precipitate, the time required to make the determination was greatly shortened. The method is presented as follows:

PREPARATION OF REAGENTS

(a) *Sodium cobaltinitrite.*—Dissolve 5.1666 grams of pure sodium cobaltinitrite in 40 cc. of distilled water. Dissolve 27 grams of potassium-free sodium nitrite in another 40 cc. of distilled water. Pour the solutions together, add 6 cc. of glacial acetic acid, and make up to exactly 100 cc. with distilled water. Theoretically 1

* By E. M. Emmert. The investigation reported is in connection with a project of the Kentucky Agricultural Experiment Station and is published by permission of the Director.

[1] *J. Am. Chem. Soc.*, 25, 990 (1903).

[2] *J. Chem. Soc.*, 77, 1076 (1900).

cc. = 10 mg. of potassium as $K_2NaCO(NO_2)_6$. (The solution evolves gas when first prepared, but this does not impair its value.)

(b) *Sodium nitrite solution.*—Dissolve 27 grams of potassium-free sodium nitrite in distilled water and make up to 100 cc.

TABLE 1.

Quantity of reagent (a) to use.

QUANTITY OF YELLOW PRECIPITATE	APPROXIMATE K PRESENT	REAGENT TO USE
Very small	0.3–0.8	0.1*
Small	0.8–1.5	0.2*
Medium	1.5–4.0	0.5
Heavy	4.0–9.0	1.0
Very heavy	9.0–19.0	2.0

* Dilute 1 cc. of reagent (a) to 10 cc. with reagent (b). Use 1 and 2 cc. respectively of this diluted solution.

(c) *Sodium nitrite solution (dilute).*—Add an equal volume of distilled water to 100 cc. of reagent (b).

Organic matter, if present in sufficient amount to interfere, or if the sample is soil or tissue, may be oxidized with sodium chlorate and nitric

TABLE 2.

Recovery of potassium in known solutions.

K ADDED mg.	K FOUND mg.	ERROR per cent	K ADDED mg.	K FOUND mg.	ERROR per cent
0.3	0.302	0.67	3.0	2.90	−3.33
0.4	0.380	−5.00	4.0	4.04	1.00
0.5	0.500	0	5.0	5.00	0
0.8	0.770	−3.75	5.0	4.90	−2.50
1.0	1.020	2.00	5.0	5.06	1.20
1.0	0.950	−5.00	7.0	7.06	0.86
1.0	0.96	−4.00	7.0	7.11	1.37
2.0	1.900	−5.00	8.0	8.04	0.50
2.0	2.010	0.50	8.0	8.08	1.00
2.0	1.990	−0.50	10.0	10.84	8.40
2.0	1.980	−1.00	15.00	15.90	6.00

and sulfuric acids as directed by Emmert.[1] Ammonia and metals that cause the solution to be colored may be removed by boiling the solution with sodium carbonate and filtering. Oxides of nitrogen may be evolved, but this does no harm if the bubbles are not allowed to accumulate under the plunger of the colorimeter.

PROCEDURE

Several portions of the unknown solution containing between 0.3 and 19 mg. of potassium are evaporated almost to dryness. Reagent (a) is added in quantities indicated by preliminary tests based on the quantity of yellow precipitate formed (see Table 1).

[1] *This Journal*, 12, 240 (1929); *J. Am. Chem. Soc.*, 25, 990 (1903).

If a series of samples containing about the same potassium content is being analyzed, one preliminary test will suffice. After 5 minutes the solution is made up to 20 cc. with reagent (c). A solution free of precipitated potassium-sodium cobaltinitrite is obtained by filtering through a dry retentive filter, by centrifugalizing, or by letting it stand not less than 3 hours. This solution is compared in the colorimeter with a solution made by making up the same quantity of reagent (a) as was used in the unknown to 20 cc. with reagent (c). The calculation of potassium from the colorimetric readings is the same as that used in the colorimetric determination of carbon dioxide.[1]

TABLE 3.
Potassium found in tomato stem extracts.
(Expressed as p.p.m. of green tissue)

PLOT	DET. 1	DET. 2	PER CENT OF ERROR FROM AVERAGE
1	1780	1780	0
2	1290	1290	0
3	2580	2510	2.7
4	2216	2216	0
5	2771	2710	2.2
6	2216	2216	0
7	830	700	16.9
8	700	700	0
9	700	930	28.1
10	1290	1180	8.9

TABLE 4.
Potassium found in soil extracts.
(Expressed as p.p.m. of air-dry soil)

PLOT	DET. 1	DET. 2	PERCENTAGE OF ERROR FROM AVERAGE
1	172	172	0
2	134	134	0
3	172	172	0
4	514	424	19.1
5	277	206	29.4
6	206	172	18.0
7	339	362	6.5
8	487	487	0
9	424	424	0
10	487	501	2.8

The results with Baker analyzed potassium nitrate are given in Table 2. Of the 22 determinations only 3 gave an error as high as 5 per cent and only 2 higher than 5 per cent, the highest percentage of error being 8.40.

The results obtained on plant and soil extracts are given in Tables 3 and 4.

[1] *This Journal*, 14, 386 (1931).

The Estimation of Esters in Distilled Liquors*

Three analysts, using the method adopted as official by the Association of Official Agricultural Chemists, returned the following proportions by weight of esters per 100,000 parts by weight of alcohol, as being present in two samples of Scotch whisky:

ANALYST	WHISKY I	WHISKY II
A	28.6	—
B	68.8	69.1
C	41.5	52.5

These differences are serious, as the proportion of esters is one of the criteria relied upon in the identification of particular brands of whisky. The method is a very simple one, and it was considered by the writer that the differences were due to an error or errors not generally recognized. As suggested by Lunge[1] it seemed most probable that the solubility of the glass in the alkali used in saponifying the esters is a disturbing factor. Therefore parallel experiments were carried out, Jena and unbranded glass flasks being used. Three samples of whisky were used, and the following results were obtained:

WHISKY	JENA FLASKS	UNBRANDED FLASKS
I	41.4	37.3
II	50.1	64.5
III	53.7	78.8

As these data show that the kind of glass used does affect the result obtained, further experiments were made upon a solution containing 32

Esters in distilled liquors.

KIND OF GLASS	PARTS BY WEIGHT ETHYL ACETATE PER 100,000 DUPLICATE RESULTS	AVERAGE	ERROR
Pyrex	49.4		
	52.2	50.8	−16.0
Jena	58.3		
	60.6	59.5	−7.3
Chance Bros	58.5		
	62.3	60.4	−6.4
Wood Bros	69.4		
	72.4	70.9	+4.1
Bohemian	68.8		
	75.7	72.3	+5.5
Schola	78.7		
	80.8	79.8	+13.0

* By F. H. Campbell (Melbourne, Australia).

[1] Lunge-Keane, Technical Methods of Chemical Analysis, (1914) III, part 2, p. 724.

per cent by volume of ester-free alcohol and 66.8 parts of ethyl acetate (boiling between 73.5° and 74.5°C.) by weight per 100,000 parts of alcohol by volume. The A.O.A.C. method was used, the only modification being that the solutions were cooled in an atmosphere free from carbon dioxide. In each case 50 cc. of the ester solution and 25 cc. of 0.1 *N* sodium hydroxide were used. The flasks were steamed before use. The results of these determinations are tabulated above.

The above data show that the results obtained in any one kind of glass are closely reproducible, but that when different glasses are used seriously divergent results may be obtained. In all cases the blank experiment was conducted in a flask of the same kind; had this not been done the differences might have been much greater, for example, had the maximum value obtained for a blank in this series and the minimum value for the ester solution been used the result would have been 136 parts of ethyl acetate, whereas 66.8 parts of ethyl acetate were present. If on the other hand, suitable glass is used, the method as modified yields results which are very satisfactory.

The A.O.A.C. method is similar to methods given in various standard text books; the exception is that it specifies a blank determination. That this is desirable is shown by the fact that in an experiment under the conditions prescribed by the A.O.A.C. the value obtained was 60.6 parts of ethyl acetate, while, had the factor of the sodium hydroxide determined in the cold been used, the value would have been 86.4 parts.

A test of a method including Lunge's suggestion and the use of alcohol in the blank was made with the stock ester solution containing 66.8 parts of ethyl acetate per 100,000 volumes of alcohol. The quantity found was 56.2 parts, as compared with 59.5 parts found in Jena flasks by the A.O.A.C. method. There is evidence that the most important cause of the difference is the use of alcohol in the blank. Parallel blanks, in which water and 32 per cent alcohol, respectively, were used, were carried out under the following conditions: 50 cc. of the liquid was boiled for an hour under a reflux condenser with 25 cc. of 0.1 *N* sodium hydroxide, cooled after the flask was closed with a stopper carrying a tube filled with Askarite, and titrated with 0.1 *N* sulfuric acid, phenolphthalein being used as an indicator. The following results were obtained:

KIND OF FLASK	cc. of 0.1N H_2SO_4 per 100,000			
	WATER	ALCOHOL	DIFFERENCE	DIFFERENCE AS ESTERS
Jena.....	23.79	23.71	0.08	1.41
Pyrex.....	23.58	23.41	0.17	3.09
Chance Bros.....	23.90	23.70	0.20	3.5

These results show that the action of the alkali on the glass is modified by the presence of alcohol and that the values obtained in the three sorts of glass used would have been still more divergent from the true value had an alcohol, instead of a water, blank been used.

BOOK REVIEW

Chemistry for Students of Agriculture and Home Economics. By ROBIN CHARLES BURRELL. 459 pages, 77 figures. McGraw-Hill Book Company, Inc., New York, 1931. Price $3.50.

The author makes the following statement: "Little distinction is usually made between courses in chemistry for the student who wants to know this science as the professional chemist should know it and for the student who merely wants to know about it as a man or woman of broad interests and culture. This text is intended for the latter."

"The book presupposes a thorough course in the principles of General Inorganic Chemistry" "Some laboratory experiments are included along with the descriptive part of the text so that the student may be better able to appreciate how a chemist works These experiments may also give a number of students an opportunity to find out that they have a talent for this kind of work and that they wish to pursue it farther"

"The book has been planned in such a way that it is adapted for use in daily two-hour periods without reference to a formal division into class days and laboratory days."

The book is divided into five parts as follows: Fundamental Principles of General Chemistry, Analytical and Synthetic Chemistry, Organic Chemistry, Biological Chemistry and Chemistry and the World's Work.

The appendix includes directions for beginning laboratory work, a list of chemical supplies and equipment, a list of group problems, convenient tables and a glossary.

The book is written in clear style, and the author seems to have accomplished his objects very well.—H. R. KRAYBILL.

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